Host–guest interactions in thin membranes: selective ion transport and transduction into electronic signals

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Abstract. Synthetic receptor molecules that selectively complex with charged guest molecules can be used to transport salts through liquid membranes and to transduce chemical information into electronic signals. In both cases the receptor molecules are present in thin membranes in contact with aqueous solutions. Extreme lipophilicity of the receptor molecules is therefore required: calix crown ethers and calixarene spherands meet these requirements. Their synthesis and complexation properties will be discussed. In order to mimic the large rates of transport through biomembranes, thin supported liquid membranes (< 100 μm), in which the receptor molecules are present, were investigated. The selective ion transport has been studied as a function of the experimental parameters and interpreted via computer simulations of the transport processes. The transduction of complexation into electronic signals can be achieved via the ‘immobilization’ of receptor molecules on the gate surface of an ISFET chip. Parameters that govern the signal transduction in multilayer systems have been studied and simulated.


The increasing interest in the design and synthesis of molecular receptors and their complexing properties with charged and neutral guests has been stimulated by the possible applications of such synthetic receptors in chemistry, medicine, and material science. In this chapter we shall describe the synthesis of receptor molecules based on calix[4]arenes and their application in two fields: selective transport of salts through liquid membranes, and the direct transduction of chemical interactions into electronic signals.

These two potential applications have been selected from the ongoing research efforts in our group. They will serve to illustrate that the translation of complexation properties at the molecular level into the desired macroscopic properties of a ‘chemical system’ or a ‘molecular material’ is not a simple extrapolation, but a scientific challenge in itself. It is not a coincidence that in
FIG. 1. Structures of macrocyclic receptor molecules; 1a-d, four different conformations of p-tert-butylicax[4]arene; 2, spherand; 3, hemispherand; 4, cryptakispherand; 6, calix crown ether.
both examples the synthetic receptors are embedded in a thin organic film, because ultimately most applications of molecular receptors will require manipulation at the nanometre level (nanochemistry), or even at the molecular level.

The first part of this chapter will deal with the synthesis of receptor molecules based on calix[4]arenes. We decided to investigate this type of building block because the calix[4]arene structure (1a–d, Fig. 1) has (i) a potential molecular cleft or cavity, (ii) four functional OH groups and (iii) an intrinsic hydrophobic character that will be needed to avoid destabilization of the ultimate chemical system in contact with aqueous solutions.

Calix[4]arenes as building blocks for synthetic receptor molecules

The calix[4]arenes are easily accessible from the base-catalyzed condensation of p-tert-butylphenol and formaldehyde, due to the pioneering work of Gutsche and co-workers (Gutsche et al 1989). Subsequent (selective) de-tert-butylation and/or reaction of one or more of the phenolic groups leads to a rapidly increasing number of different calix[4]arenes. Gutsche proposed to define the two faces of the calix molecules as the lower (phenolic groups) and upper rims; we shall use this nomenclature here. The calix[4]arenes with free OH groups are conformationally flexible and the molecules can adopt four different extreme conformations (1a–d in Fig. 1). When the phenolic groups are alkylated or acetylated with a substituent larger than methyl, the interconversion of the different conformations is no longer possible. Recently we have found that tetramethylcalix[4]arenes are mixtures of conformers in which all four forms can be found (L. C. Groenen, unpublished work 1990).

We became interested in calix[4]arenes as building blocks in relation to the synthesis of a receptor for a radioactive isotope of Rb+, which can be used for organ imaging (Van Herk & De Zeeuw 1978). Since in biological fluids Rb+ will readily exchange with Na+ and K+ ions, only a kinetically stable complex of Rb+ can be used. The spherands (2) (Fig. 1) reported by Cram & Lein (1985) form kinetically stable complexes with Na+ and Li+ but they reject K+ and larger alkali cations completely. Hemispherands (3) or cryptahemispherands (4) (Fig. 1) do not form kinetically stable complexes in aqueous solutions (Dijkstra et al 1988, 1989) because the cations are not sufficiently shielded from the solution. Consequently, we designed a host molecule that combines the shielding ability of the spherands with the cavity size of the cryptahemispherands, by combining a calix[4]arene and a terphenyl bridge to form a calixspherand (5) (Fig. 2) that forms kinetically stable complexes with Na+, K+, and Rb+ ions (M+) (Dijkstra et al 1989). The half-life of decomposition at room temperature varies between different cations from one year to two hours and the high kinetic stability is attributed to the partial cone conformation (1b) of the calix[4]arene moiety in the 5.M+ complex, which was observed both in the solid state (see Fig. 2) and by 1H NMR spectroscopy in solution.
5. $M^+$

FIG. 2. The X-ray-derived structure of the complex of an alkali cation ($M^+ = \text{Na}$) and a calixspherand (5).

Systematic variation of the bridge between O(2) and O(4) oxygen atoms of the 1,3-dimethoxy-calix[4]arene moiety yielded a series of receptor molecules that exhibit extremely high $K^+ / \text{Na}^+$ selectivities. These receptors (6) (Fig. 1) have been used for the membrane transport experiments that are described in the second part of this chapter and for the selective transduction of complexation reactions into electronic signals, described in the third part. We have found that the selectivity of complexation can be further enhanced by increasing the preorganization (Cram & Lein 1985) of the binding sites in the calix crown ethers (6) (Fig. 1). This was achieved by alkylation of 6 ($R = \text{H}$) with more bulky alkyl groups ($R = \text{C}_2\text{H}_5$, n-$\text{C}_3\text{H}_7$, i-$\text{C}_3\text{H}_7$, CH$_2$C$_6$H$_5$; Ghidini et al 1990). The different conformers—cone, 1,3-alternate and partial cone (Fig. 1)—exhibit different selectivities and thermodynamic stabilities of complexation with different cations.

Receptor-mediated transport of salts through supported liquid membranes

The most remarkable observation by Pedersen (1967) in his studies on crown ethers was the shape-selective complexation of alkali cations—that is, that the stability of the complex is dependent on the size of the cation. Not surprisingly, the use of this property in selective separation of cations via membrane transport
was one of the first potential applications of these compounds to be investigated. Until recently, all experiments with this aim in mind were carried out in bulk liquid membranes (Lamb et al 1980) composed of two aqueous phases separated by a solution of the receptor molecules in an immiscible organic solvent. Although important results on the selectivity and rate of transport were obtained, the frequently mentioned analogy with biological membranes is hardly realistic (Stolwijk et al 1987).

We have therefore studied a system which is much closer to the dimensions of biomembranes, the supported liquid membrane system. The receptor molecules, dissolved in an organic solvent, are immobilized in a thin (micro)porous polymer membrane \(d = 100 \mu\text{m}\). The rate of transport of different salts through such membranes has been studied as a function of a number of parameters of the system (Stolwijk et al 1989a). The initial studies revealed that the partition coefficient of the receptor molecule is extremely important (Stolwijk et al 1989b), together with the association constants of the complexes formed, the diffusion coefficient of the complex, and the partition of the salt between the aqueous and the membrane phases (source phase and receiving phase; Fig. 3). We can now describe the membrane flux for single ion transport with a model based on these independently determined parameters (Fig. 3).

Generally, such membrane systems are not stable because the receptor leaches out of the very thin membrane. In order to improve this stability, molecular properties, other than those of complexation, have to be introduced into the structure of the receptors. The calix crown ether (illustrated in Fig. 1) shows a large selectivity for K\(^+\) in comparison with Na\(^+\) and, when it is substituted with four \(p\)-tert-butyl groups, its partition coefficient increases to \(>10^5\), sufficiently large to retain the receptors in the membrane for days. An alternative to the use of \(p\)-tert-butylcalix crown ethers is the covalent attachment of polysiloxanes to the receptor molecules (Wienk et al 1990).

When we studied the selectivity of the K\(^+\)/Na\(^+\) transport through these supported liquid membranes with \(o\)-nitrophenyl octyl ether as the solvent, we observed a much lower selectivity than was expected on the basis of the complexation data for Na\(^+\) and K\(^+\) in chloroform (W. F. Nijenhuis, unpublished work 1990). A computer simulation of the entire transport process as a function of all parameters, that predicts the selectivity, has been developed.

Our results demonstrate that the extrapolation of molecular complexation properties to macroscopic properties, in this case selective membrane transport, requires a great deal of fundamental research.

Direct transduction of chemical information into electronic signals

In biological systems, selective interactions between organic hosts and (in)organic guests are used to store and transduce chemical information in order to maintain
and replicate the species. Consequently, an important goal in host–guest (supramolecular) chemistry for the next decade will be to generate molecular systems that are able to transduce chemical interactions at the molecular level into observable quantities at the macroscopic level. Far-reaching ideas of extending supramolecular chemistry to larger assemblies with such functions have been outlined by Lehn (1988) in terms of ‘nanochemistry’ (10^-9–10^-7 m). The application of such systems as molecular electronic components will require extension up to the ‘nanochemistry’ level and also a way to transduce molecular properties to the macroscopic level.

We have decided to follow a different approach for reaching these molecular systems, by integrating synthetic receptor molecules with a semiconductor chip.
This approach takes advantage of the ability to manufacture devices at the sub-micron level ($\geq 3.5 \times 10^{-7}$ m) (Santo & Wollard 1988). As the transducing element we have chosen a field-effect transistor and for the sensing element we have used a variety of macrocyclic polyethers and calix crown ethers. We have focused our attention on the integration of both the sensing and the transducing elements in one molecular system that can be regarded as a chemical entity with a designed function (chemically sensitive field-effect transistor, or CHEMFET).

A field-effect transistor (FET) is able to register the conductance of a semiconductor as a function of an electric field perpendicular to the SiO$_2$ gate oxide surface (Fig. 4). Bergveld (1970) demonstrated that the conductance of a FET is influenced by the interface potential at the oxide/aqueous solution because of the (de)protonation of silanol groups at the SiO$_2$ surface. He then showed that chemical information from the solution was transduced into an electronic signal that can be processed by the microchip.

We decided to use this FET to study direct transduction of complexation reactions between hosts and guests into electronic signals by attaching receptor molecules covalently to the SiO$_2$ gate surface of a FET. This required first the elimination of an undesired pH dependance by covalent modification of the SiO$_2$ surface (van den Berg et al 1985). A monolayer was not sufficient for this purpose, so we have focused on the covalent attachment of thin (10–25 μm) polymer layers to the ‘gate’ surface. Silylation with 3-(methoxyisilyl)propyl methacrylate or 3-(triethoxysilyl)propylamine gave reactive surfaces that were subsequently modified by co-polymerization with acrylate monomers and prepolymer of polyurethane, respectively. These covalently attached layers

**FIG. 4.** Schematic representations (top and side views) of a field-effect transistor (FET) in which S, D, and B are the electrical contacts for measuring the relative electrical potentials. (1) electrical reference; (2) the insulating SiO$_2$ layer (gate); (3) the resin that insulates the devices from the solution; (4) the conducting channel between the two contacts S and D.
almost completely eliminated the pH sensitivity of the microchip. When small amounts of anionic species, always present in these polymers, are neutralized with small amounts of lipophilic cations, these devices are insensitive not only to pH but also to the ionic strength of aqueous solutions. This gives a reference FET (REFET) which is a prerequisite for the potentiometric measurement of chemical interactions (Skowronska-Ptasinska et al. 1990).

The complete shielding of the SiOH groups by the covalently attached polymers has one disadvantage. The equilibria at the interface are no longer thermodynamically defined, because a common species (H⁺ or OH⁻) is lacking. This gives rise to unstable electrical potentials. We have solved this problem in the following way. The SiO₂ surface is first reacted with 3-(methoxysilyl)propyl methacrylate. The bulk of this reagent limits the effective silylation of the SiOH groups to 1.5 functional groups per nm² and consequently 3.5 SiOH groups per nm² surface will remain. Subsequent photo-

copolymerization with 2-hydroxyethyl methacrylate (HEMA) covers the SiO₂ surface with covalently attached polyHEMA (layer thicknesses between 5 and 15 μm). The polyHEMA was subsequently saturated with a buffered KCl solution to give a hydrogel. This means that at constant local pH the residual SiOH groups fix the interface potential of the FET (Sudhölter et al. 1990).

The hydrogel was reacted with methacryloyl chloride in order to attach covalently reactive methacryloyl groups, and a polysiloxane membrane containing methacrylate groups was covalently attached (Fig. 5). The structure of this polysiloxane membrane, which will ultimately contain the receptor molecules, has to meet very distinct requirements for the transduction of host–guest interactions to the FET device.

We have optimized the glass transition temperature (Tg) and the dielectric constant (ε) by systematic variation of the ratio of the different siloxane building blocks and we have introduced receptor molecules that selectively complex alkali cations (van der Waal et al. 1990) (Fig. 5). Again it proved to be important that the receptor molecules are sufficiently hydrophobic, in order that they should not leach out of the thin membranes that are covalently linked to the chip. These membranes have a distinct upper limit of thickness; too thick layers isolate the FET electrically. Calix crown ethers showed excellent transduction of variable concentrations of K⁺ in the range of 10⁻⁴–10⁻¹ M, even when 10⁻¹ M of NaCl was present. These results demonstrate that our first objective—the covalent integration of molecular receptors and a FET into a chemical system (CHEMFET)—has been achieved. This molecular system connects the chemical and the electronic domains (Reinhoudt & Sudhölter 1990). Computer simulations of the entire system are in progress.

The first practical applications of our approach to the transduction of complexation reactions into electronic signals are chemoselective sensors. When such a sensor was incorporated into a flow injection device we were able to measure K⁺ concentrations in biological fluids in the millimolar range.
FIG. 5. Schematic representation of the chemical substructures of a membrane covalently attached to the SiO₂ gate surface of a field-effect transistor. HEMA, hydroxyethyl methacrylate.

These two examples of how complexation properties of receptor molecules can be extrapolated to macroscopic properties, (the transport of salts through liquid membranes, and the transduction of chemical into electronic signals) demonstrate that this process requires more than just creating larger assemblies of receptor molecules.

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Host-guest interactions in thin membranes


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DISCUSSION

Albery: You showed a diagram of your MOSFET (metal oxide field-effect transistor) device (Fig. 4) in which the metal contact was replaced by the solution. In that diagram the device was very big but you illustrated the reference electrode as very small. I imagine that the sizes are the opposite way around, with the
reference electrode being quite large; or have you miniaturized the reference electrode?

Reinhoudt: For the electronics field, that is the important question. We were not the first to use FETs, but I think we were the first chemists to integrate the whole system covalently so that it has a long life-time. The reference electrode is made in exactly the same way as the CHEMFET. We remove the pH sensitivity of the original silicon dioxide in exactly the same way as we do for a CHEMFET, by covering it with a series of polymer layers, but leaving out the ionophore. On top of that there is a delicately balanced equilibrium between hydrophobic anions and hydrophobic cations. It has been recognized only recently that in most polymers there are some anionic sites that are introduced during the synthetic process by an emulsifier or a starter, for example. By high resolution secondary ion mass spectrometry we have shown that anionic sites are present in all polymers that we have investigated so far, even PVC (van den Berg et al 1987). Because of this, there will be a response to changes in ionic strength. You can eliminate that effect almost completely using a small amount of a quaternary ammonium cation and a long alkyl chain which has a high partition coefficient.

Albery: What determines the potential of that reference electrode?

Reinhoudt: That's a mystery. Both the anions and the cations will determine the potential, but when you combine their effects they compensate each other to give a potential of almost zero over a useful range of ionic strength.

Albery: That is most mysterious.

Reinhoudt: This, for us, is the most difficult thing to understand. When you model the whole system in order to find out how the potential is built up at the interface, you find that on changing from an immobilized anion to an immobilized cation there should be a sharp change from cation to anion selectivity. I think the easiest way to view the reference electrode is as a combination of a cation- and an anion-selective electrode, with the same number of anionic and cationic sites.

Stoddart: You described the anchorage as being of a covalent nature. Did I misunderstand you? Surely, the calix crown was non-covalently attached in some way to the polysiloxane. If it was, what is the nature of that interaction? Does the difficulty of attaching the calix crown covalently limit the device's life-time? Do you plan to attach it covalently?

Reinhoudt: The covalent attachment I referred to is of the membrane system (the hydrophilic membrane, the intermediate layer and the hydrophobic membrane) to the ISFET chip. There has been much debate about the possibility of linking the carrier molecules to the hydrophobic membrane. You can model the potential build-up at the interface because the diffusion potential is a result of the mobility of all the species involved, assuming there has to be a thermodynamic equilibrium. If you immobilized the ionophore it would have zero mobility and, theoretically, there would no longer be a potential. But what
Host-guest interactions in thin membranes

is zero mobility? Could you attach the ionophore via a long chain and still maintain some mobility? We have done some experiments involving covalent attachment not of the calixarene, but of a calcium-selective ionophore. We see a normal Nernstian response of 30 mV per decade when this ionophore is covalently attached to the membrane. The attachment is made by copolymerization in the final stage of preparation of the hydrophobic membrane.

Although the calix crown is not covalently attached, our K⁺ sensor works for at least six months because the partition coefficient of the calix crown is about 10⁴, so it is retained in the membrane. 18-Crown-6 or a cryptand would be retained in the membrane no longer than minutes.

Gokel: You said that your molecules were highly selective for K⁺ over Na⁺, but that the selectivity assessed in o-nitrophenyl octyl ether did not correspond to that determined in chloroform. Do you have any idea how the selectivity of your molecules compares with that of valinomycin, the selectivity of which is known in a number of media? Also, why did you choose to use a calixarene rather than valinomycin, when valinomycin is probably more selective and probably equally hydrophobic (although it might prove quite hard to attach covalently)?

Reinoudt: Valinomycin is about three times more selective than the calix crown. My estimate of valinomycin's selectivity in chloroform is about 10⁴:1. We have also tested valinomycin in a liquid-immobilized membrane transport experiment (Stolwijk et al 1989). There the K⁺/Na⁺ selectivity is about 60:1.

Gokel: What was the selectivity of the calixarene there?

Reinoudt: It was 23:1.

Gokel: So valinomycin is about three times more selective in that situation also.

Reinoudt: The difference in selectivity is related to the transfer energy of both ligands (K⁺ and Na⁺) between one solvent and another.

Gokel: Why do you not use valinomycin?

Reinoudt: We began this work with the aim of making a completely stable, covalently attached system. It's not easy to covalently attach valinomycin to the hydrophobic membrane. I wouldn't say that it would be impossible to construct a device with valinomycin that has almost the same response as one with a calixarene, but although the initial response of valinomycin at very low K⁺ concentrations is a little better than that of the calixarenes, in the relevant concentration range (10⁻³–10⁻²) it is not.

Ron Breslow: In your experiment you were changing a DC signal to an AC signal. There are a number of things that you can do with AC that you can't do with DC, such as studying phase relationships and non-linear responses. Do you think you will go on to use an AC rather than a DC signal?

Reinoudt: I am not a solid-state physicist but I understand more or less how the ISFET works. The ISFET measures a change of the membrane potential and that change is transduced as a difference in the current in the channel, which
is immediately compensated for by a change in the potential. You keep the
current constant by changing the potential and that’s what you actually record
(see Fig. 4).

Albery: If you go over to using an AC rather than a DC technique, the kinetics
of the system will become significant. At the moment, you don’t need to bother
about your rather curious thermodynamics. I think the problems that this would
cause would outweigh the advantages.

Ron Breslow: Problems are a good thing!

Reinhoudt: We have tried to measure the response rate of the CHEMFET,
but we could not record the build-up of the signal quickly enough to see whether
the rate of complexation is the rate-determining step. We have never been able
to follow the process of signal build-up; it reaches the maximal level almost
immediately.

Albery: Does it happen over a microsecond time-scale?

Reinhoudt: I don’t think we can measure this in membranes, but it’s faster
than 0.1 second.

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