Synthetic Ion Channels

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Solid-State Ion Channels for Potentiometric Sensing**

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Biological ion channels (ICs) are protein pores with amino acid sequences providing functionalities rigorously spaced in the pore lumen to induce selective recognition and passage of ions through the cell membrane.[1] For example, the K^+ ion is at least 10^4 times more permeant through K^+ channels than the Na^+ ion.[2] Protein engineering and chemical modifications offer the possibility to further enhance and diversify the molecular recognition properties of biological nanopores,[3] in particular their ion selectivity.[4] Apart from their biological significance and emerging sensing applications,[5] selective ion channels may certainly have a use in ion separation, for example in desalination[6] and cleanup of radioactive ions.[7] However, industrial-scale applications are limited, among other things, by the intrinsic fragility of the lipid bilayers and membrane proteins. Theoretical modeling studies suggest that by analogy to the selectivity filter of biological ICs, synthetic solid-state ion channels (nanotubes, nanopores) may also be constructed if their lumen is controllably modified with proper functionalities.[8] However, despite the successful use of nanopores modified with selective receptors for enantioselective[9] and DNA transport,[10] the selectivity of ion transport through nanopores has generally been based only on charge repulsion,[11,12] size exclusion,[13] or polarity.[14] But these nanopores discriminate between groups of compounds having widely different physicochemical properties rather than providing selectivity for given species.

Herein, we introduce for the first time solid-state ICs based on ionophore-modified nanopore arrays and, as a first application, their use for potentiometric sensing of a small inorganic ion. We used gold nanopores formed by electroless deposition of gold onto the surface of polycarbonate track-etch membranes with randomly distributed straight cylindrical pores (6×10^8 pores cm^{-2} with nominal diameters between 15 and 80 nm).[11] This arrangement allows for the spontaneous self-assembly of thiol- and disulfide-bearing ionophores and other selectivity-tuning compounds in a monomolecular layer within the nanopores. Moreover, the gold plating restricts the effective diameter of the nanopores in order to have their chemically modified inner surfaces govern the transport. The proposed solid-state construction overcomes the fragility of biological ICs, while it also has the potential to relieve major limitations of conventional ionophore-based liquid-membrane ion-selective electrodes (ISEs), which constitute the foundation of the blood electrolyte analyzer industry.[15] Such electrodes usually comprise membranes made of highly plasticized PVC[16] incorporating the ionophore, ion-exchanger, and other lipophilic additives. Any of the membrane components can leach into the sample solution, which limits the lifetime of the electrodes[17] and restricts their applicability. While efforts have been made to overcome these limitations by using self-plasticizing polyacrylate-based membranes,[18] as well as by covalently confining active ingredients to the polymer matrix[19] or nanoparticles,[20] there is still no complete solution to this problem. Moreover, not only leaching of ion-selective membrane (ISM) components into the sample is detrimental; owing to their selectivity-altering effects, the extraction of lipophilic sample components such as neutral lipids from human body fluids into the polymer membrane is also of concern.[21] This extraction of lipophilic components can be avoided only by using superhydrophobic fluorous-polymer-based ISMs,[22] which, owing to their extremely poor solvation capacity, resist the extraction of highly lipophilic components. However, on the downside, the poor solvation makes such ISMs incompatible with commercially available ionophores and impedes their general applicability.

Herein, we propose a solid-state ISM configuration with all components immobilized by Au–S bonds onto the walls of Au nanopores. This approach is radically different from recent efforts in nanoscaling potentiometric sensors, which only focus on a reduction in size of conventional membrane materials.[23] For proof of principle, a synthetic Ag^+-selective thiacalixarene derivative bearing dithiolane moieties (SS-Ag-II, Scheme 1) was used to induce Ag^+ selectivity.[20]

The length of the Au nanopores (6 μm) is approximately three orders of magnitude larger than the length of biological pores, and it approaches the thickness of conventional polymeric ISMs (ca. 100 μm). The theory of ionophore-based ion-selective membranes predicts that membranes of finite thickness, as in our case, require negative sites to induce a proper potentiometric response.[24] Consequently, cation-exchanger sites were generated using mercaptodecanesulfonate (MDSA), while, to take advantage of the latest results showing the superiority of fluorous ISMs,[22] a perfluorinated thiol derivative (PFT) was used to confer hydrophobicity to the Au nanopores (Scheme 1).

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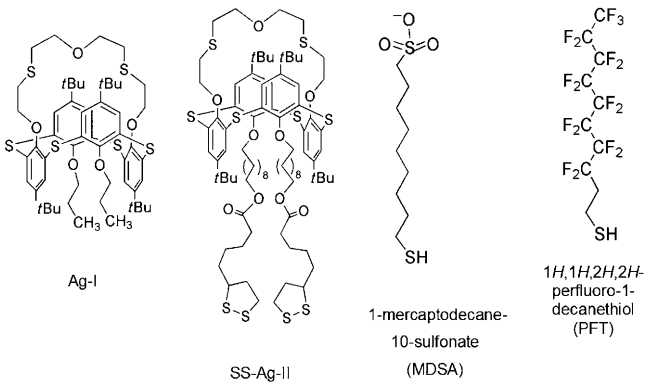

Scheme 1. Components of conventional plasticized PVC (Ag-I) and nanopore-based ISMs (SS-Ag-II, MDSA, PFT).

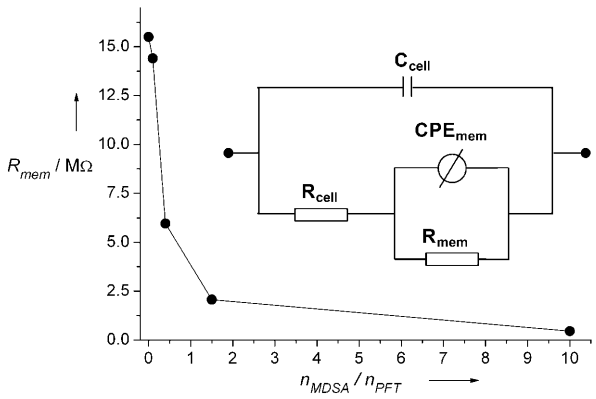

Figure 1. Change in the membrane resistance upon modification of the Au nanoporous membranes (1.9 nm effective pore radius) with MDSA/PFT mixtures of various molar ratios for 30 min. The inset shows the equivalent circuit used to calculate the membrane resistance.

In a first step, Au nanopores were prepared by electroless Au plating for 300 min[25] within the pores of track-etch polycarbonate membranes having 15, 30, and 80 nm pore diameters. The establishment of cation permselectivity was considered the first criterion to determine the proper pore size. The cationic permselectivity of the native and MDSA-modified Au nanoporous membranes was tested using commercial Philips electrode bodies[26] with 1 mM CsCl as inner solution and changing the CsCl concentration in the sample in the range of 10^{-4} to 10^{-2} M. To achieve cation permselectivity, the inner walls of the pores should be negatively charged and the pore diameters should be within the range of the Debye length. Thus, the establishment of the permselectivity becomes more critical with larger pore diameters and higher ionic strength solutions.[11] Indeed, while for MDSA-modified Au nanopores with smaller initial pore sizes of 15 and 30 nm, the membrane potential changed linearly versus $\log a_{Cs^+}$ (where a is the activity) in a nearly Nernstian manner, the electrode function of membranes with 80 nm initial pore size deviated from linearity (Supporting Information, Figure S1). After performing N_2 permeation experiments and calculating the effective pore radii of the Au nanopores within the membrane by the Knudsen equation,[27] we found indeed significantly larger effective pore radii (13.6 ± 0.3) nm for the 80 nm than for the 15 and 30 nm initial-pore-diameter membranes ((1.9 ± 0.5) nm). Therefore, for further optimizations we used Au-plated 15 or 30 nm initial-pore-diameter track-etch membranes with effective Au nanopore diameters of less than 5 nm. The pore diameters were then further restricted to molecular dimensions by subsequent modification with the thiol derivatives.

We used electrochemical impedance spectroscopy (EIS) to gather evidence for the confinement of the various components within the nanopores. The complex plane impedance plots of modified Au membrane (Supporting Information, Figure S2) revealed two arcs. The high-frequency semicircle corresponds to the resistance and capacitance of the transport cell without the Au membrane, while the low-frequency arc corresponds to the membrane resistance and a capacitive element, that is, a constant phase element (CPE; Figure 1 inset).

The EIS technique is particularly sensitive to the modification of the nanopore surface with hydrophobic compounds, but it is less efficient for detecting hydrophilic modifications. For instance, the resistance (R_{mem}) of the Au membranes of less than 45 kΩ (initial pore diameter 15 nm; $A = 0.196$ cm^2) changed to 15.5 and 1.2 MΩ upon modification with PFT and ionophore, respectively, but it did not alter significantly upon modification with MDSA. To provide evidence for binding of MDSA into the nanopores, the Au membranes were treated with methanolic solutions of MDSA and PFT in various molar ratios with a fixed total concentration of 0.1 mM. The competitive nature of the formation of mixed self-assembled monolayers was expected to decrease R_{mem} as the MDSA molar fraction in the modifying solution increases. These expectations were confirmed by the experimental results as shown in Figure 1, which consequently indicates the formation of a mixed self-assembled monolayer within the nanopore lumen.

The challenge at this stage was to find the proper ratio of the surface-modifying thiol- and dithiolane-functionalized compounds to confer Ag^+ selectivity to the nanoporous ISMs. The obvious prerequisite for ion selectivity is to have the ionophore in a molar excess with respect to the negative sites in the mixed self-assembled monolayer within the nanopore. Remarkably, successive modifications of the Au nanoporous membranes with thiols having markedly different polarities, such as subsequent treatment of MDSA-modified membranes with either PFT or SS-Ag-II, did not cause any increase in the membrane resistance. Therefore, the Au membranes were modified in one step with all components dissolved in methanol. As contact-angle experiments performed on planar Au surfaces (Supporting Information, Figure S3) suggest that MDSA binds to Au at significantly lower ratios than its molar ratio in the modifying solutions, a mixture of SS-Ag-II/MDSA/PFT at molar ratios of 11:10:1 and a total concentration of 0.2 mM was used for subsequent functionalizations. To determine the potentiometric response of the ionophore-modified nanoporous membranes, they were mounted in Philips electrode bodies[26] with 0.1 mM $AgNO_3$

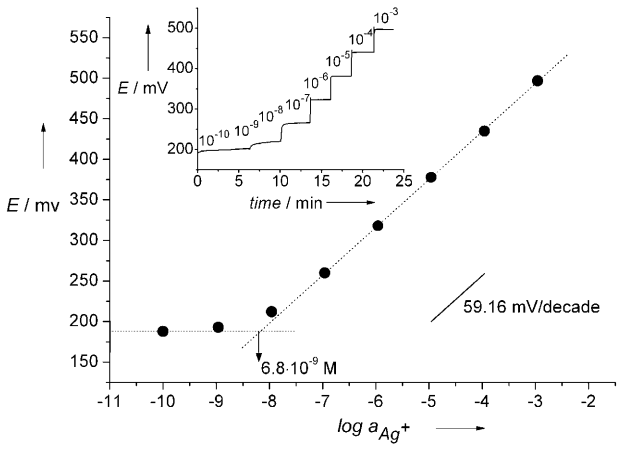

Figure 2. Calibration curve of an ionophore-modified Au nanopore-based Ag^+-selective electrode. Membranes with 3 nm effective pore diameters were modified with a mixture of ionophore/MDSA/PFT at molar ratios of 11:10:1 and total concentration of 0.2 mM for 20 h. The inset shows the corresponding potential trace.

as internal solution. The potentiometric Ag^+ response of optimized nanopore-based ISEs is shown in Figure 2.

Detection limits in the lower nanomolar concentration range associated with fast Nernstian potential responses were determined after no special conditioning. Of note is the drift-free potential response, which indicates that the ion transport through the nanopores must be negligible under zero-current conditions. Furthermore, excellent selectivities exceeding six orders of magnitude were determined for a range of representative interfering cations (Table 1). In contrast,

Table 1: Potentiometric selectivity coefficients $\log K^{pot}_{Ag,J}$ of nanopore-based ISEs with and without ionophore modification.

Interferent (J)	SS-Ag-II/MDSA/PFT (11:10:1)	MDSA/PFT (10:1)
K^+	−6.1 (−4.9,[20] −7.5[28])	−1.0
Cs^+	−6.5	−0.9
H^+	−6.0 (−7.4)[28]	−0.2
Et_4N^+	−6.5 (−4.3)[28]	−1.2

nanopores modified similarly, but without the ionophore, hardly showed any Ag^+ selectivity. The selectivities for K^+ and H^+ lag behind those determined with Ag-I ionophore-based PVC membranes,[28] but the beneficial effect of the fluorous nanopore membrane is obvious when considering the significantly better selectivity for quaternary ammonium ions. Such lipophilic organic cations with high affinity for hydrophobic phases are ubiquitous interferents of the most commonly used solvent polymeric membranes. As expected, this interfering effect is considerably reduced in the PFT-modified nanopore-based ISEs. The PFT modification was also found to be essential for proper potentiometric response and ion selectivities, thus suggesting the importance of hindering the access of water into the nanopores.

In conclusion, using the selectivity filters of biological ICs as inspiration, we have reported for the first time ionophore-modified solid-state nanopores exhibiting extraordinary ion-recognition selectivities. This achievement required adjusting the hydrophobic, ion exchange, and selective ion complexation properties within Au nanopores of less than 5 nm effective diameter by modifying their surface with a mixed self-assembled monolayer consisting of three different thiol derivatives having distinct functionalities. Potentiometric transduction is introduced as an exceptionally simple means to demonstrate the ion-selective behavior of modified nanopores with immediate practical applicability for ion sensing. The new ISE construction preserves the exquisite selectivity of ionophores in a robust solid-state membrane format with all active components covalently immobilized. Therefore, Au nanopores seem to offer a versatile platform to integrate various ionophores and other functional components of markedly different properties, given that they possess thiol or disulfide functionalities, and by that to enable the selective recognition of a wide range of ions. However, the concept of inducing ion selectivity is likely to be extendable to other type of nanoporous materials and pore geometries, which beyond sensing might find application in ion separation and characterization of relevant host–guest interactions.

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