Sensing DNA Hybridization via Ionic Conductance through a Nanoporous Electrode

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We show that nanoporous alumina modified with covalently linked DNA can be used to detect target DNA by monitoring the increase in impedance at the electrode upon DNA hybridization, which resulted from blocking the pores to ionic flow. Using cyclic voltammetry, direct current conductance, and impedance spectroscopy we confirm the importance of pore size: the effect is observed with 20-nm-diameter pores and is absent for 200-nm pores.

Malfunction of ion channels in biological cells leads to alteration of a trans-membrane potential and can cause cell death. Understanding of such important fundamental biological phenomena, as well as the possibility to control ion flow, attracts great interest in the study of ionic transport through nanopores. Recently, the idea of controlling the mass transport of specific species through nanopores by means of UV light[1], pH of solution[2], charge,[3,4] and size[5] of an ion was explored. Biosensors and separation membranes that are based on selective nanopores have evolved into a very promising field.[6−8] Technological advances in the past decade have made it possible to manufacture nanopores with dimensions comparable to the sizes of biological polymers such as short DNA and peptides. Some groups take advantage of such an approach and use single nanopores to resolve sequences of individual DNA molecules linked to a degree of partial pore blockage by the DNA.[9] To our best knowledge, control of the ion current through nanopore arrays by DNA hybridization inside the nanopores has not been demonstrated.

Here we explore this alternative approach and investigate variation of ionic conductivity in nanopores caused by DNA immobilization at the pore walls and its subsequent hybridization with a complementary DNA strand. We believe that this method could also be employed with other biological objects which possess strong enough bioaffinity interactions.

In our previous work, we have shown that alumina nanoporous membranes with 200-nm-diameter pores can be used for DNA separation and optical detection by hybridizing with single-stranded DNA (ss-DNA) covalently immobilized inside the pores.[8] Here we present investigation of electrical manifestation of the hybridization event, namely, altering the ionic current through alumina nanoporous membranes by immobilized ss-DNA upon its hybridization with target DNA. Electrical detection is more desirable because of possibilities of miniaturization, integration into existing detection schemes, and realization of parallel arrays.

Cyclic voltammetry (CV), time dependence of direct current (dc), and impedance spectroscopy with $Fe(CN)_6^{4-/3-}$ and $Ru(NH_3)_6^{2+/3+}$ redox pairs were used to probe the efficiency of ion blockage. These redox pairs were chosen because of their relatively large size, reversible electrochemistry, and differing charges.

The procedure of DNA immobilization inside nanopores was previously described[8] and consisted of three steps: silanization with aminosilane, activation with glutaraldehyde, and covalent attachment of 5′-aminated DNA oligomer (21-mer in our case). We took advantage of commercial anodized aluminum oxide (AAO) filter membranes, which have a nominal 200-nm diameter through most of $L = 60$ μm thickness except for approximately 1 μm on one side that has a smaller diameter, 20 nm in this case (see Supporting Information).[4] By choosing an orientation of either the 200-nm side or the 20-nm side toward the working electrode (see Scheme 1) investigation of the size effect on hindering the ionic conductance by immobilized and hybridized DNA becomes possible. The pores on the small diameter side are comparable to the DNA length, nearly 7.5 nm for a double-stranded 21-mer, and, thus, are expected to affect the conductance the most. Orienting the 200-nm side toward the working electrode should make no difference on the ionic conductance because of incompatibility of pore and DNA sizes, if the measurements are performed on the time scale shorter than diffusion through the whole membrane thickness. Hybridization with target DNA was performed outside the cell, and its efficiency was tested by optical absorption.[8]

A flat platinum working electrode was placed in close contact with the tested side of a membrane, while a screen counter electrode was in contact with the opposite side (see Scheme 1). Such geometry minimizes contribution from the solution outside the membrane, which is mostly distinguishable at high frequencies. CV in Figure 1A clearly shows that both reductive and oxidative currents

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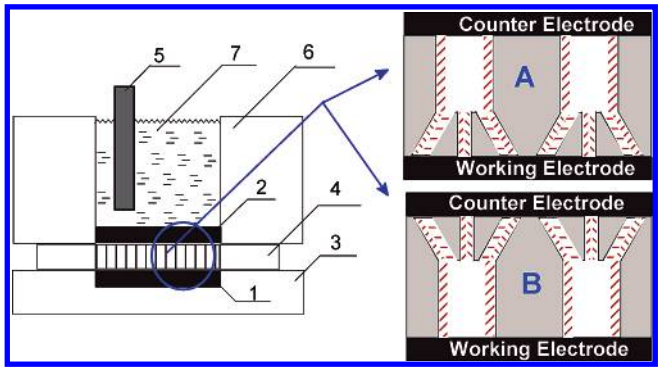
Scheme 1[a]



[a] Two options for filter (4) orientation [A, working electrode (1) at the 20-nm side of the membrane; B, at the 200-nm side] in the homemade electrochemical cell with a stainless steel screen counter electrode (2) and reference minielectrode (5), immersed in solution (7). The working electrode is made of Pt, and the cell body (3 and 6) is made from Plexiglas.

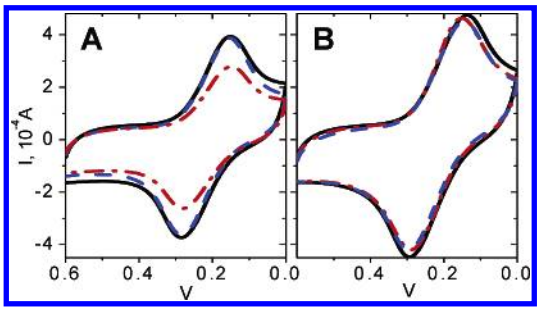


Figure 1. CV in the region for $Fe(CN)_6^{3-/4-}$ oxidation/reduction: (A) at the 20-nm side of a modified membrane and (B) at the 200-nm side. Solid black lines, 21-mer ss-DNA immobilized inside pores; red dashed−dotted line, DNA hybridized with a complementary 21-mer strand; and blue dashed line, after denaturing with 9 M urea. Voltage versus Ag/AgCl; 100 mV/s; 10 mM $Fe(CN)_6^{3-}$/10 mM $Fe(CN)_6^{4-}$ in 0.1 M KCl.

decrease after DNA hybridization occurs inside the nanopores and when the 20-nm side is oriented toward the working electrode. The scan rate of 100 mV/s was sufficiently high so that the thickness of the diffusion layer was shorter than the membrane thickness. As expected, no noticeable current change was observed at the 200-nm side of the same filter (Figure 1B) because the length of double-stranded DNA (ds-DNA) is much smaller than the pore diameter. The higher current amplitude on the 200-nm side is primarily due to a higher ion mobility through larger pores, but a possible difference in effective areas of contact with the working electrode can have a small contribution as well.[4] The CV amplitude did not change on either side when a noncomplementary target DNA was used, which agrees with the lack of hybridization, also confirmed by optical measurements.[8] The current amplitude on the 20-nm side recovers to its original value upon DNA denaturing with 9 M urea, while no effect was observed again for the 200-nm side. Because of a small Debye length, $\lambda_D = 0.307/c^{1/2}$, in 0.1 M KCl (~1 nm),[11] no specificity to the ion charge is anticipated. Indeed, similar results were obtained with the positively charged $Ru(NH_3)_6^{2+/3+}$ redox pair (see Supporting Information). A change in conductivity can also be observed in dc, as shown in Figure 2, where variation of the charge due to reduction of $Fe(CN)_6^{3-}$ traveling through the filter (at the 20-nm side) is given as a function of time. The accumulation of

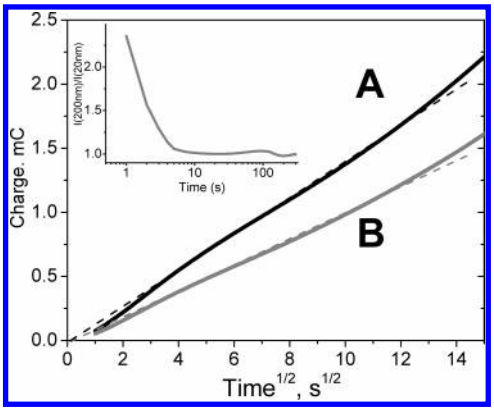


Figure 2. Chronocoulometric plot for charge passed through the modified nanoporous membrane modified with 21-mer ss-DNA immobilized inside the pore filter before (A) and after (B) hybridization with the complementary 21-mer. The working electrode is at the 20-nm side in both cases. Other conditions are similar to those in Figure 1: 10 mM $Fe(CN)_6^{3-}$/10 mM $Fe(CN)_6^{4-}$ in 0.1 M KCl; 0 V versus Ag/AgCl. The inset illustrates the ratio between the currents through unmodified membrane oriented by the 200-nm side, I(200 nm), and the 20-nm side, I(20 nm), toward the working electrode.

charge appears proportional to the square root of time, from where the net diffusion coefficient, D, can be estimated from the known concentration of $Fe(CN)_6^{3-}$ and the electrode area, A, using eq 1:[11]

$$Q(t) = 2AF[Fe(CN)_6^{3-}](Dt/\pi)^{1/2} \quad (1)$$

where F is the Faraday constant.

The net value of D, measured at long times in Figure 2, drops from $\sim 1.1 \times 10^{-6}$ cm²/s in the case of immobilized ss-DNA to $\sim 6.2 \times 10^{-7}$ cm²/s upon DNA hybridization. This estimate agrees with measurements of ion flux through the filter (see Supporting Information) and previously reported observations on unmodified filters.[4] The diffusion coefficient of $Fe(CN)_6^{3-}$ is nonuniform through the filter thickness, but on the time scale exceeding the diffusion time through the wide part of the membrane, t_D, the anisotropy in its orientation diminishes. As the inset in Figure 2 shows, the anisotropy declines within about 6 s, in agreement with the estimated diffusion time through 200-nm portion of the membrane:[11]

$$t_D = L^2/4D \quad (2)$$

During the time t_D of electrolysis, nearly half of the ferricyanide ions from inside the membrane are reduced at the working electrode. Similarly in CV measurements, the anisotropy is large at fast scan rates and declines with its lowering.

Information on ion flow is conveniently illustrated through the frequency dependence of cell impedance, Z, measured at the half-wave voltage for the redox pair (0.22 V vs Ag/AgCl). Figure 3 shows this in the form of a Nyquist plot for $-\mathrm{Im}\ Z$ versus $\mathrm{Re}\ Z$ in the frequency range from 10^5 to 10^{-2} Hz. Again, the impedance appears higher when the filter is oriented with the 20-nm side toward the working electrode and *increases* when ss-DNA inside the pores is hybridized with a complementary oligomer of target DNA. The 200-nm side is practically unaffected by DNA hybridization. Because the modulation voltage in impedance measurements is small, the anisotropy effect between the 20-nm and the 200-nm sides persists over a broad range of frequencies.

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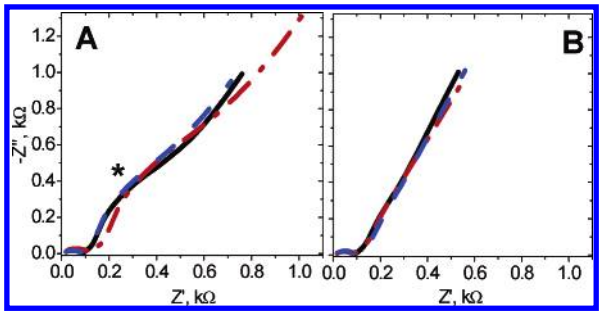

Figure 3. Nyquist plot for impedance at 0.22 V (vs Ag/AgCl) for the same filter and conditions as in Figure 1. (A) 20-nm side of a membrane, (B) 200-nm side. Solid black lines, 21-mer ss-DNA immobilized inside the pores; red dashed−dotted line, immobilized DNA hybridized with a complementary strand; blue dashed−dotted line, after denaturing with 9 M urea.

Table 1. Effect of the Immobilized DNA Surface Density Inside AAO on Its Impedance Change at 0.01 Hz at the 20-nm Side upon DNA Hybridization

	$\Delta\|Z\|$ at 0.01 Hz	
N_Ω, cm^{-2}	complementary[a]	noncomplementary[b]
1×10^{12}	75%	<3%
8×10^{11}	33%	n/a
6×10^{11}	23%	n/a

[a] The complementary 21-mer as in ref 10 shows a 93% hybridization efficiency inside the pores as measured by optical absorption. [b] The noncomplementary 21-mer as in ref 8 shows a less than 3% hybridization efficiency inside the pores as measured by optical absorption.

The described effect of conductance change in nanopores has a complex nature with contributions from: (a) volume exclusion due to additional DNA upon hybridization, (b) the ionic mobility change inside the pores entangled with charged DNA strings, and (c) the distribution of pore diameters. This complexity is revealed in the initial *decrease* of impedance after immobilization of ss-DNA inside the nanopores, as compared to an unmodified filter. In contrast, the impedance always *increases* (amplitude of CV *decreases*) upon hybridization of already immobilized DNA with the complementary oligomer. The changes are observed exclusively on the 20-nm side. Similarly, the ratio between the CV amplitude on the 20-nm and that on the 200-nm side first increases from the initial value of 0.74 for the unmodified filter to about 0.87 upon immobilization of ss-DNA but then drops to about 0.62 after DNA hybridization (see Supporting Information). The impedance increase is dependent on the surface coverage of immobilized ss-DNA and the degree of its hybridization with target DNA, as illustrated by Table 1 and Figure 4. The impedance increases nonlinearly with the surface concentration of ss-DNA upon "complete" hybridization (approximately 93% as judged by optical absorption) with the complementary 21-mer. Quantitative analysis of this dependence should be taken with great caution because the concentration measured by optical absorption is accurate only for the 200-nm portion of the filter, which covers most of its 60-μm thickness and DNA; the concentration at the 20-nm side can differ for either the ss-DNA or the ds-DNA case.

The described geometry is not convenient for real time monitoring of the hybridization effect and analysis of the concentration dependence of the signal. However, it allows for unambiguous discrimination of the pore blocking effect from various phenomena that alter rates of redox reactions at the flat electrode.[10] On the basis of the data presented, the effect of the hindered ion mobility inside modified nanopores, the size of which is comparable to the DNA length, is clearly confirmed. Figure 3 suggests that the mobility drop can be quite dramatic. The inflection point in the Nyquist plot at the 20-nm side is observed in the vicinity 0.1 Hz (on the Warburg portion of the plot of Figure 3, as well as in the Bode plot, Supporting Information). This should correspond to the diffusion coefficient on the order of 10^{-7} cm^2/s, if one assigns the inflection point frequency to the inverse time of ion traveling through 1 μm of modified pore. This diffusion coefficient is almost 2 orders of magnitude smaller than that in the bulk solution, but the value is consistent with the estimates based on optical measuring of the ion flux through the membrane (see Supporting Information). Although some dependence on the ion charge might be expected if lower ionic strengths were used, for 0.1 M KCl this effect disappears (see Supporting Information) in agreement with previous observations.[4]

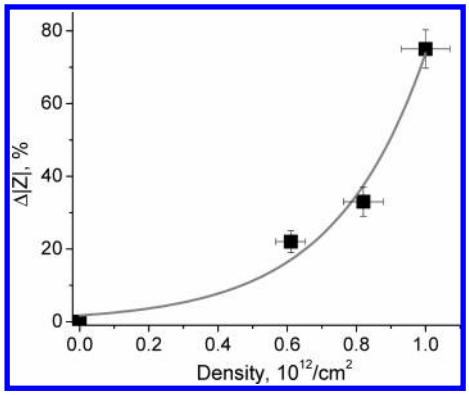

Figure 4. Impedance increase at 0.01 Hz upon complete DNA hybridization as a function of surface density of initial ss-DNA. The solid line serves as a guide for eye.

Thus, nanoporous alumina with covalently linked ss-DNA on its surface can be used for electrical detection of complementary target DNA sequences without need for their modification. Electrical detection should offer a greater versatility in miniaturization, integration into inexpensive detection schemes, and realization of parallel arrays, that is, DNA chip. Better understanding should be achieved with uniform distribution of nanopore diameter and length. The suggested approach is far from being optimized, but the theoretical sensitivity limit can be conservatively estimated using our experimental data by taking electrodes of 5 μm × 5 μm area and 0.5-μm-thick oxide pores of 20-nm diameter. In that case, less than 10^{-17} mol of complementary target DNA is required to hybridize surface-immobilized ss-DNA with the same density, 10^{12} cm^{-2}, as in the described cell and achieve a more than 70% impedance increase, as shown here for similar densities. We are progressing toward experimental realization of this estimate and hope to achieve even better sensitivity.

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Supporting Information Available: (a) Information on sequences used, (b) procedure for electrochemical measurements, (c) ss-DNA surface density and (d) hybridization efficiency calculations, (e) scanning electron microscopy images of the filters, (f) measurements of ion flux through the membrane, (g) electrochemical measurements with the $Ru(NH_3)_6^{2+/3+}$ redox pair, and (h) variation of the CV ratio between the 20-nm and 200-nm sides as a function of filter modification. This material is available free of charge via the Internet at http://pubs.acs.org.

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