"Direct" Detection and Separation of DNA Using Nanoporous Alumina Filters

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The concept of using alumina nanoporous filters (AAO) modified with DNA for "label-free" detection and separation/purification of the target ss-DNA is demonstrated. The high surface density of DNA (4×10^{12} cm^{-2}) and high efficiency of hybridization (ca. 70%) in combination with increased effective surface area make this system very attractive for development of various ss-DNA (or RNA) detection methods. Moderate transparency of AAO in the UV and IR regions allows direct detection of DNA hybridization by optical and IR absorption. Close to the quantitative efficiency of binding the target ss-DNA from solution using a single pass through the modified filter is observed.

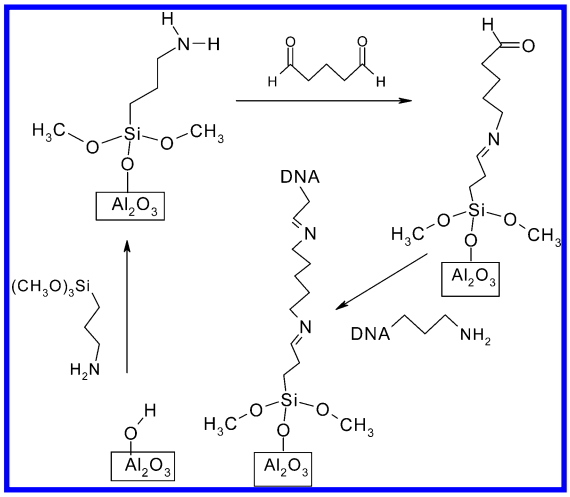
Utilization of bioaffinity interactions is an indispensable tool of modern biochemical research. Bioaffinity interactions such as DNA−DNA and antigen−antibody are employed for identification of the presence of a particular DNA sequence in a sample, for detection and identification of microbial and viral species, and for verification of efficacy and function in medical diagnostics. Specificity of these interactions can be also employed for purification.

Detecting ss-DNA fragments by utilizing their hybridization with complementary sequences immobilized on a surface is at the heart of the DNA chip technology. Various methods have been employed for identification of the hybridization event: fluorescence,[1] surface-enhanced Raman,[2] surface plasmon resonance,[3] interferometric,[4] and others.[5] Currently, the method of choice is fluorescence detection because of its high sensitivity, but it requires modification of the DNA. There is an apparent need for more direct methods of DNA/RNA detection that are inexpensive and reliable and would not require tagging of DNA with fluorescent dyes. Detection by optical absorption (either UV or IR) is usually not considered because of its low sensitivity. However, with an increased surface area density, the advantage of direct detection by absorption should become more attractive.

In this "proof of concept" report we demonstrate that modified nanoporous alumina filters can be used for DNA detection and separation. Here we utilize UV and IR absorption for direct detection of unmodified DNA, but other detection techniques can be also applied.

Aluminum anodized oxide disks (AAO) with their fairly well-defined nanopores have become popular in various applications. High pore density ($1 \times 10^9/cm^2$) and small pore diameters (20−200 nm) result in a substrate with high surface area that can be easily functionalized. The effective area of a 60-μm-thick filter with 200-nm pores corresponds to about 1000 greater density of surface sites, that is, a 1000-fold increase in optical density for the same density of a DNA monolayer on the surface.

Scheme 1



Similar to the methods of glass and silica surface modification, hydroxyl groups on the alumina surface can also be used for chemical functionalization. Because of the lower acidity of alumina surfaces and spatial hindrance of nanopores, the surface modification chemistry has to be revisited. After evaluating different methods,[6] we found that the most reliable approach that ensures a high density of covalently linked DNA inside the nanopores is achieved with a glutaraldehyde linker which joins the amino groups of 5′-aminated DNA and the terminal amino group of aminosilane, as shown in Scheme 1. Unreacted glutaraldehyde is neutralized by propylamine.

Figure 1 illustrates that, using this approach, high optical densities (OD ∼ 1) can be reliably obtained with ss-DNA 21-mers on AAO filters. Higher loadings, OD > 1.6, are easily achievable but are inconvenient for UV absorbance measurements with an ordinary spectrom-

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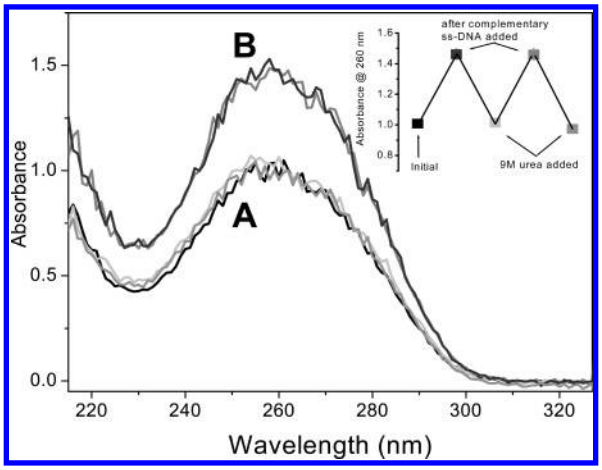

Figure 1. UV absorption spectra of a 200-nm AAO filter (60-μm thick) with (A) ss-DNA 21-mer immobilized inside the pores and (B) after hybridization with a complementary 21-mer. The inset illustrates the reversibility and reproducibility of hybridization and denaturation (with 9 M urea).

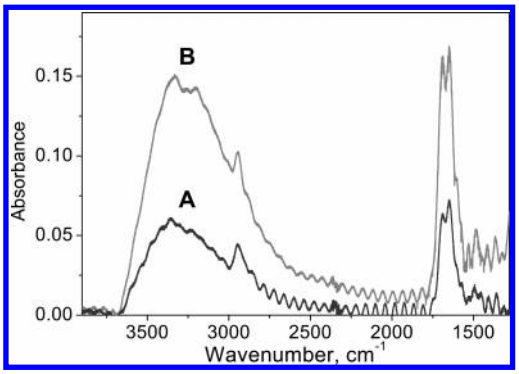

Figure 2. (A) IR absorption of a 200-nm filter with immobilized 21-mer and (B) after its hybridization with a complementary 41-mer.

eter.[7] Similar results were obtained with smaller pore diameters of (20 nm) AAO. The absorbance of OD = 1.0 from Figure 1 corresponds to the DNA surface density of about 2.6×10^{12} cm^{-2}. Upon hybridization with the complementary 21-mer, the UV absorbance increases to 1.45, which accounts for about 70% hybridization efficiency. The hybridization efficiency decreases to 50% when no glutaraldehyde neutralization was performed. A similar hybridization efficiency was obtained with a complementary 41-mer. The high hybridization efficiency is corroborated by the infrared (IR) spectra shown in Figure 2, where a 2-fold absorption increase in the regions of in-plane double bond[8] vibrations near 1700 cm^{-1} and in the hydrogen region around 3300 cm^{-1} are observed. As a result of background variations we could not assess the conformation (B or A) of the hybridized DNA. No increase of either UV or IR absorption was observed with a noncomplementary ss-DNA (21-mer or 41-mer) on the same filter. The bound complementary ss-DNA can be eluted by either using denaturing solutions, as shown in Figure 1, or by heating the filter in water; the procedure can be repeated numerous times without noticeable loss of the surface-immobilized ss-DNA. Fluctuations in the absorption are due to slight variation of the filter placement in the cuvette. It can be minimized in a flow cell configuration.

(7) The apparent optical density of a clean filter with 200-nm pores in water at 260 nm is about 2. That is the primary reason for working with less than maximum loading.

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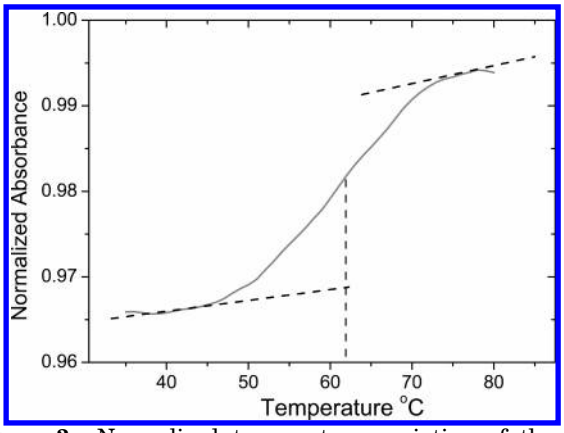

Figure 3. Normalized temperature variation of the UV absorption at 260 nm for the immobilized hybrid between the 21-mer and the 41-mer of curve B in Figure 1 in 0.1 M NaCl.

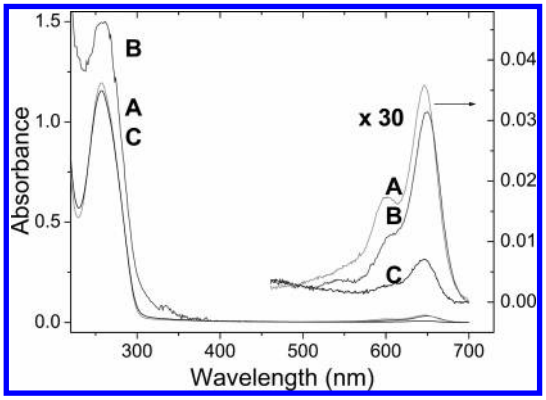

Figure 4. Absorption spectra of 1 mL of solution originally with 185 nM of Cy5-tagged ss-DNA (21-mer) before (A) and after a single pass through the AAO affinity filter (C). (B) Spectrum of the filter with hybridized Cy5-tagged complementary ss-DNA.

Figure 3 provides additional confirmation that the increase in UV absorption is due to DNA hybridization. The melting temperature observed for the immobilized hybrid, T_m = 62 °C, is very close to that measured in solution. The small amplitude of the apparent hypochromism on the filter is due to dilution into a larger volume.

The AAO filter with covalently immobilized DNA can be also used as an affinity separation tool for specific target ss-DNA. A solution is simply passed through the filter, and the bound target ss-DNA is then eluted in the purified form by denaturing the hybrid at an elevated temperature or by using denaturing solutions. We demonstrate this on a filter with 200-nm pores, on which 3.0 nmol of ss-DNA was immobilized (OD = 0.75 for the 21-mer used). When an excess (6 nmol) of the complementary ss-DNA (also a 21-mer) in 0.1 M NaCl was slowly passed through that filter at room temperature (25 °C), 1.6 nmol of the target ss-DNA was captured. This corresponds to almost the same filter capacity as estimated above using the 41-mer. The captured DNA was eluted with >90% efficiency by a 9 M urea solution, as observed by UV absorption spectra changes of the filter and the solutions.

When a mixture of 6 nmol of noncomplementary 21-mer ss-DNA and 0.3 nmol of the complementary 21-mer labeled with the Cy5 dye at 5′ was passed through the same filter, 85% of the complementary ss-DNA was captured, while the noncomplementary ss-DNA did not bind, as shown in Figure 4.

In summary, we have shown that alumina nanoporous filters can be successfully employed to immobilize DNA using aminosilanes and glutaraldehyde linker. The high surface density of DNA ($\sim 4 \times 10^{12}$ cm^{-2}) and high efficiency of hybridization (ca. 70%) in combination with high surface area make this system very attractive for further development of various DNA/RNA detection methods. The moderate transparency of AAO in the UV and IR regions allow direct detection of DNA hybridization by optical absorption without any modification of the target ss-DNA. The standard approach with fluorescent dye-tagged ss-DNA target can also take advantage of the AAO filter through the high density of immobilized ss-DNA and stability to thermocycling, combined with excellent transparency in the visible range.

The unique advantage of using the AAO filter is in a convenient combination of detection and separation/purification possibilities for unmodified target ss-DNA (and RNA). Close to quantitative efficiency of binding the complementary DNA (>80%) from a solution by a single pass through the modified filter and a low interference from noncomplementary ss-DNA make this a promising method for various applications.

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Supporting Information Available: Detailed procedures and calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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