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Fabrication of DNA micropatterns on the polycarbonate surface of compact discs

Zhen Wang · Rong-Xian Li

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Abstract DNA micropatterns have been fabricated on the surface of Polycarbonate (PC) bases of compact discs, with the ultimate goal of using conventional CD technology for DNA analysis. To confirm the formation of -COOH groups on the PC surface upon UV/ozone treatment, contact angle titrations were carried out on the PC surfaces. The surface morphologies of PC surface were measured by Tapping-mode Atomic Force Microscopy (AFM). As the surface as ''anchor'' to attach the amine-modified ssDNA by covalent conjugates via amide bonds, the results of fluorescence spectroscopy showed that the fluorescein labeled complementary ssDNA can be used for detection the ssDNA immobilized on the patterned CD polycarbonate substrate.

Keywords DNA · Compact disc · UV/ozone · Hybridization

Introduction

DNA microarrays have attracted increasing interest due to the many benefits of device miniaturization and parallel gene analysis. They are generally fabricated on glass, silicon, or metal surfaces [1, 2]. Synthetic polymers are promising alternative substrates because of their low specific gravity, high elasticity, and low cost. In the past, nylon membranes have been used to make DNA microarrays, but their applications are limited as they exhibit lateral wicking characteristics and the attached DNA labels tend to spread from the points of immobilization [3]. The recent developments of these techniques include in-depth statistical analyses of the robustness of, for example high density arrays spotted onto glass slides and the differences between slides supplied from at least three commercial sources. Therefore, considering to take a specific problem associated with expression analysis of a biological system and performing a comparative quantitative assessment of CD based arrays to define the advantages over other established approaches.

Polycarbonate (PC) is an important thermoplastic because of its high optical clarity, tensile elongation, and impact strength. It is the base material for the commercial manufacturing of compact discs (CDs) that are prepared by an inexpensive injection molding protocol. Aside from being popular information storage media, CDs have proven to be versatile for modern chemical research [4]. Initial studies in this area were focused on the fabrication of biosensing devices on circular plastic disks, which integrate microfluidic functions with the CD technology, e.g., the control of fluid transfer by disk spinning and sample analysis with the optical detection system [5, 6]. The idea of using CD technology to detect biorecognition reactions was proposed by Hammock and co-workers in 2000 [7]. Remacle et al. created unique bio-CDs, storing both numeric and genomic information, as platforms for gene analysis [8]. They coated the CD surface with an additional polymer fixation layer prior to DNA immobilization. La Clair and Burkart reported a protocol to screen protein binding to selected ligands attached to CDs [9]. However, it is cumbersome to control the

Z. Wang (✉) · R.-X. Li
Center for Advanced Materials & Biotechnology, Research Institute of Tsinghua University in Shenzhen, Shenzhen 518057, P.R. China
e-mail: wangzhen919@126.com

activation of PC surface as it involves phosphorylation with dichloro-N,N-diisopropyl aminophosphate in harsh organic solvents [9].

In the past, the activation of polymer surfaces has relied primarily on ultraviolet (UV) irradiation. Liu et al. treated PC with a manual UV lamp (4 W, 254 nm) to improve the aqueous fluid transport in thus prepared microchip capillary electrophoresis devices [10a] and to facilitate the DNA probe attachment in microfluidic array channels [10b]. Welle and Gottwald studied the physico/chemical effects of deep UV irradiation (low pressure UV lamp, 15 W at both 185 and 254 nm) of PC and other polymers with respect to cell adhesion in vitro [11]. Concurrently, McCarley et al. developed a ''mild'' UV activation protocol (15 mW/cm^2 at 254 nm) to treat PC and poly(methyl methacrylate) (PMMA) for the preparation of polymer-based microanalytical devices [12a], which significantly improved their previous method [12b].

Herein we explore the feasibility of preparing DNA micropatterns on the surface of PC bases of CDs, with the ultimate goal of using conventional CD technology for DNA analysis. This is different from the previous work of using CDs for materials chemistry research, e.g., the fabrication of self-assembled monolayers [13a] and selective deposition of microstructured inorganic oxide thin films [13b]. Helt et al. [14a] and Hazarika et al. [14b] used CD substrates for patterning material nanostructures; Angnes and co-workers carried out innovative electroanalytical studies with CDtrodes (electrodes prepared from CD-Rs) [15].

Figure 1 schematically shows the three steps of our experimental approach: surface activation/patterning, attachment of DNA probe strands, and hybridization / detection of DNA target strands. The PC base of CDs was first treated in an UV/ozone cleaner to generate carboxylic acid groups. We used transmittance electron microscopy (TEM) grids as photomasks to achieve surface activation and micro-patterning in a single step [12a, 16]. To attach amine-terminated DNA probe strands, amide linkages were formed via a 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC)/N-hydroxy-succinimide (NHS) coupling reaction.

Experimental section

Chemicals and biomaterials

1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide(water soluble carbodiimide, EDC) and N-hydroxy succinimide (NHS) were both purchased from Sigma-Aldrich (St. Louis, MO) and used as received. 2-(N-Morpholino) ethanesulfonic acid (MES) was from Fluka (Buchs, CH) and used without purification. Sodium chloride, Tris and magnesium chloride were purchased from Caledon Laboratories Limited (Georgetown, ON) and used as received. All solutions were prepared with Deionized water (>18.3 MΩ cm) from a Barnstead EasyPure UV/UF compact water system (Dubuque, IA). The 0.1 M MES buffer at pH 6.5 was use for immobilization of DNA on the activated PC surface. For DNA hybridization, 10 mM Tris buffer/0.1 M $MgCl_2$/1 M NaCl at pH 7.4 was used.

Amine-modified 20-mer single stranded oligonucletides used as probes in the experiments presented: 5′-Amine-C6-CGC CGA TTG GAC AAA ACT TAA A-3′; 5′-Amine-C6-CGC CGA TTG GAG AAA ACT TAA A-3′ (mismatch); 5′Amine-C6-TTT AAG TTT TGT CCA ATC GGC G-3′ (non-complementary probe) as well as the labeled 22-mer complementary target with the sequence: Fluorescein-5′-TTT AAG TTT TGT CCA ATC GGC G-3′ were all ordered from Sigma-Genosys (Oakville, ON).

The transparent CD-ROM plates (CDs) were provided by Millennium Compact Disc Industries Inc. (Vancouver, BC). There are two different surface morphologies on the both sides of CD. One is flat polycarbonate surface, which is used in our experi-

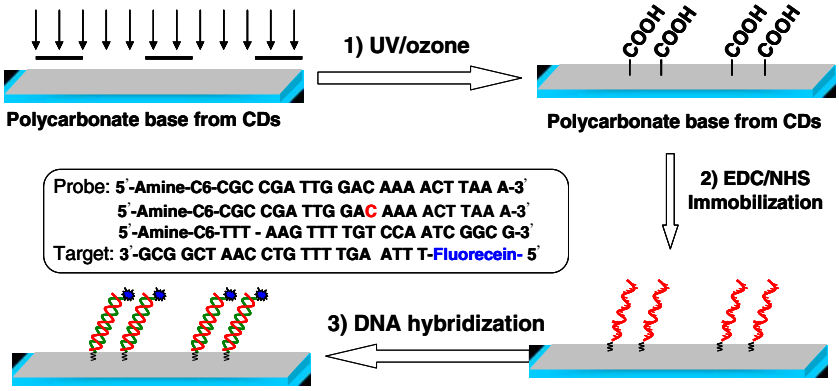

Fig. 1 Schematic diagram for DNA detection on the PC base of CDs. (1) Formation of carboxylic acid groups on the PC surface by UV/ozone treatment; (2) immobilization of amine-terminated DNA probe strands; (3) hybridization of fluorescein-labeled complementary target strands with the immobilized DNA probes

ment; another one is the surface containing the pits and lands with recorded information. TEM gold grid (G1000HSG, Pelco international) was used as mask with ca. 6 μm diameter wires with a center-to-center spacing of 25 μm.

Activation and DNA immobilization of CD polycarbonate substrate

The transparent CDs were firstly cut into the desired size plates. Without any pretreatment, these plates were directly put into the chamber of UV/O_3 Surface Decontamination (PSD/UV Novascan technology, USA) and irradiated for 10 min through TEM grid as mask on it. After the cycle is completed, the samples were continually incubated with power off in ozone environment for 30 min. Taking out the substrates from the UV/ozone chambers, we immediately spread the solution of 10 μl 10 μM single-stranded DNA (ssDNA) in 0.1 M MES buffer at pH 6.5, containing 5 mM EDC and 0.33 mM NHS on the patterned polycarbonate surface of CDs for 2 h at 20 °C under ambient conditions. After incubation, the PC substrates modified with ssDNA were washed with 0.01 M MES and deionized water, then blow-dried under N_2 gas. By forming the covalent conjugates via amide bonds, the amine terminated ssDNA was attached onto the surface of patterned PC substrate. For the part of hybridization of complementary ssDNA strand with the ssDNA immobilized on the PC substrate, the solution of 10 μl 12 μM fluorescein-labeled complementary ssDNA in buffer solution (0.1 M $MgCl_2$ and 1 M NaCl in 10 mM Tris buffer) was spread on the ssDNA-modified polycarbonate surface by heating to 90 °C, then cooling down slowly to room temperature.

Instrumentals

Fluorescent images of the DNA immobilized micropattern on the CD polycarbonate substrate were taken from the Fluorescence mode of Zeiss LSM-410 Laser Scanning Confocal Microscopy. The instrument is equipped with an Argon/Krypton laser that emits light at 488, 568 and 647 nanometers and a video camera accessory.

Tapping-mode Atomic force microscopy (AFM) images were acquired in air with Scanning Probe Microscope (SPM) (Veeco, Woodbury NY).

Contact angles between water and activated polycarbonate surface were preformed on an AST Optima contact angle system at ambient condition (22–26 °C, 43 ± 3% relative humidity) using a horizontal light beam to illuminate the liquid droplet. The contact angles measured here are thermodynamic equilibrated values of sessile liquid drops of either pure water or buffer solution. For the contact angle titration, the activated polycarbonate samples were immersed in the buffer solution for 30 s before the contact angle was measured. The exact pH values for the buffer solutions were recorded before and after the contact angle measurement.

The SEM images of surface morphologies of TEM grid as mask were taken by FEI Strata DB235 SEM/FIB (FEI Company, Hillsboro, OR).

X-ray photoelectron spectra were recorded by using a Leybold MAX-200 X-ray Photoelectron Spectrometer. The monochromatic Al Kα excitation source was operated at 1486.7 eV and 20 mW. Survey scan and narrow scan were obtained at the pass energy at 192 eV and 48 eV, respectively. The samples were mounted onto a holder with a double-sided adhesive tape and placed in a vacuum to degas.

Results and discussion

Surface activation of PC by UV/O_3 treatment

The major advantage of the UV/ozone treatment over previously reported UV irradiation (without ozone) methods is its efficiency: it takes less then 10 min to produce high density of reactive carboxylic acid groups. Conventional UV irradiation at 254 nm of PC to generate a hydrophilic surface takes much longer [10–12].

The dependence of contact angle of PC surface on irradiation time was presented in Fig. 2. The untreated polycarbonate surface of disc is hydrophobic and the contact angle was 88° ± 2°. PC surface hydrophobicity decreased with increased UV/ozone irradiation time. After the PC was exposed by UV/ozone for 2 min, the contact angle was dropped to 54° ± 2° and displays the hydrophilic surface. While the samples were kept irradiated longer than 10 min, there is no obvious change in contact angle of the surface. For the control experiment, we also applied the photochemical reactor to treat the CD polycarbonate surface by UV irradiation at 254 nm. The contact angle between water droplet and PC surface is just decreased to 55° ± 2° after 8 h UV irradiation. Compared with this phenomenon, the method of UV/ozone irradiation displays the high efficiency in photo-oxidation of polycarbonate substrate. During the procedure of the activation of PC, the irradiation time of 10 min was applied to use in the experiment.

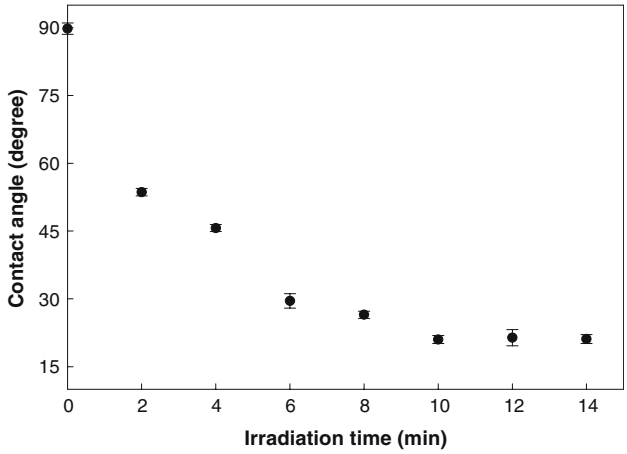

Fig. 2 The dependence of contact angle of the PC surface on UV/ozone irradiation time

To confirm the formation of -COOH groups on the PC surface upon UV/ozone treatment, contact angle titrations were carried out on the PC surfaces. Figure 3 shows a typical titration curve (open circles), in which the contact angle of buffer solutions went through a smooth transition when the pH was raised from 4 to 9, corresponding to the ionization of surface carboxylic acid groups [17]. The formation of negatively charged carboxylate anions ($-COO^-$) makes the surface more hydrophilic. In contrast, untreated surfaces (solid circles) display a constant contact angle over the entire pH range tested.

In accordance to the contact angle experiment, the information of oxidized groups at the polycarbonate surface by UV/ozone treatment was obtained by the X-ray Photoelectron Spectroscopy (XPS). The three different samples: untreated PC substrate, UV/ozone irradiated PC substrate with mask and UV/ozone irradiated PC substrate without mask were characterized in this experiment. The characteristic C 1s and O 1s XPS spectra of these different samples were shown in Figs. 4 and 5, respectively. The C1s spectra of untreated CD polycarbonate substrate have the binding energies of about 284.6 eV which were arising from the carbon in the benzene ring and the alkyl substitute. When the PC substrate was exposed in UV/ozone, the appearance of distinct high binding energy at about 288.4 eV, attributed the carboxyl group (-COOH), indicates the presence of surface activation under UV/ozone treatment. During the process of the UV/ozone treatment, TEM grid as mask was set on the surface of the CD polycarbonate. Obviously, the patterned surface area exposed to UV light is less than the surface without mask under UV/ozone irradiation. The intensity of C 1s at 284.6 eV signal for the polycarbonate surface decreased gradually after UV/ozone irradiation. Oppositely, the intensity of O1s signal with binding energy at 533.2 eV increased when the PC substrates were exposed to the UV/ozone, which is resulting from the oxidized group (-COOH) formed by UV/ozone irradiation.

Under UV/ozone treatment, the surfaces were also characterized by tapping-mode AFM. Figure 6 shows the AFM images of an untreated (a) and a treated PC surface (b). Both pictures show tracks of polishing on the mold caused by the injection molding procedure in the process of CD manufacture. The surface roughness has some change after the UV/ozone irradiation. We also note that the CDs are still *readable* in CD drives after UV/ozone treatment.

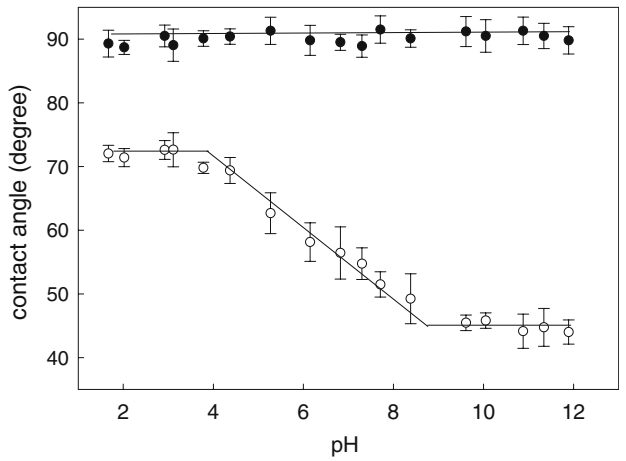

Fig. 3 Contact angle titration curves of the untreated PC surface (●) and of the PC surface treated with UV/ozone for 10 min(○)

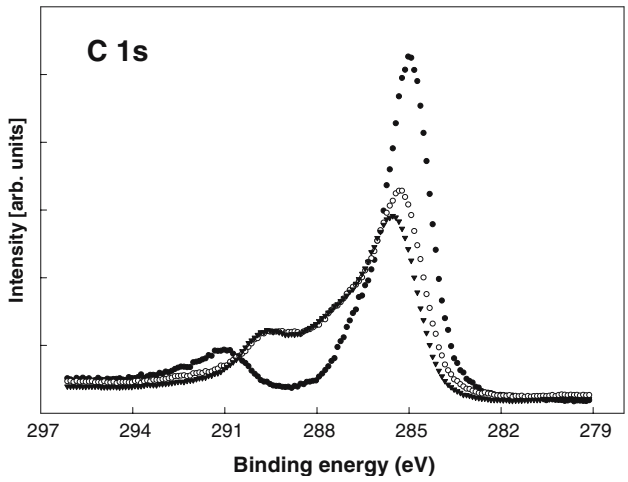

Fig. 4 X-ray photoelectron C 1s signals of the untreated polycarbonate (●) and after the UV/Ozone irradiation for 10 min with mask on (○) and without mask (▼) on the surface

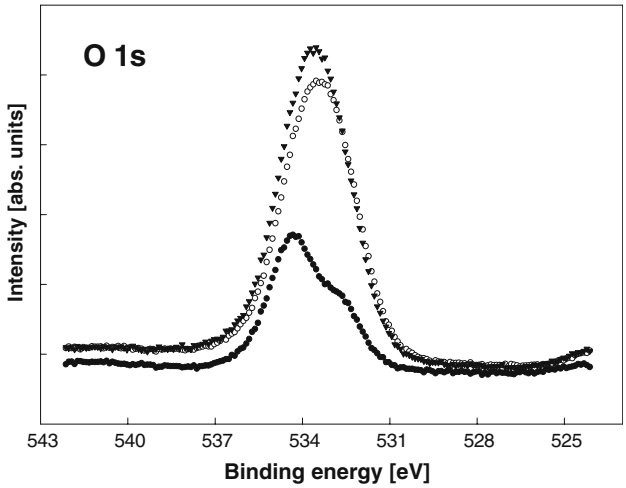

Fig. 5 X-ray photoelectron O 1s signals of the untreated polycarbonate surface (●) and after the UV/Ozone irradiation for 10 min with mask (○) and without mask (▾) on the surface

Immobilization and hybridization of DNA on activated PC surface

Contact angle titration and XPS measurements have confirmed that there are carboxylate groups formed on the PC patterned surface under UV/ozone irradiation. Upon generation of carboxylic acid groups, we then convert old CD surfaces into effective platforms for the construction of DNA microarrays by coupling with amine-terminated DNA probe strands.

The MES buffer solution is used in the carbodiimide procedure to attach the amine-termined DNA on the activated polycarbonate surface. Since the Tris containing amine group will couple with the carboxylate group of polycarbonate via carbodiimide blocking procedure. Molecules with reactive amino or carboxyl groups may be coupled to form covalent conjugates via amide bonds under using water soluble carbodiimides e.g., 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC). N-Hydroxysuccinimide (NHS) reacts with carboxyl group to give aminoacyl esters under facial condition[18]. The stable, active esters hydrolyze slowly in aqueous media compared with their rates of reaction with amino groups and can enhance the coupling efficiencies of carbodiimides for conjugating carboxylated compounds with primary amines.

As the surface as ''anchor'' to attach the amine-modified ssDNA by covalent conjugates via amide bonds, the fluorescein labeled complementary ssDNA can be used for detection the ssDNA immobilized pattern CD polycarbonate substrate.

Figure 7a shows that there is no pattern visible in the fluorescence image after the immobilization of DNA probe strands. In contrast, upon hybridization with the complementary fluorescein-labeled DNA target strands, distinct patterns are observable by fluorescence microscopy. The fluorescence pattern in Fig. 7b matches well the dimensions of the TEM grids used in the irradiation/patterning step. It should be noted that the areas exposed to the UV/ozone treatment show high signal levels, indicating the presence of fluorescein-labeled DNA strands. The unexposed areas (blocked by the ring part of a the TEM grid) did not show significant fluorescence signal, indicating that no DNA strands were immobilized at these locations. It also shows that the non-specific adsorption of DNA strands to PC surfaces is negligible.

As control, single-base mismatched and non-complementary amine-modified DNA probes were tested using the same procedures. We have shown that the fluorescein-labeled complementary DNA targets hybridize with the DNA probes with single-base mismatch immobilized on the PC surface to some extent (Fig. 7d); however, hybridization experiments with non-complementary probes showed no obvious patterns in the fluorescence image (Fig. 7c), demonstrating the high selectivity of hybridization reactions of DNA targets to the probe strands immobilized on the activated PC substrates of CDs.

Conclusion

In summary, we have prepared a novel plastic platform for the fabrication of DNA microarrays by using PC

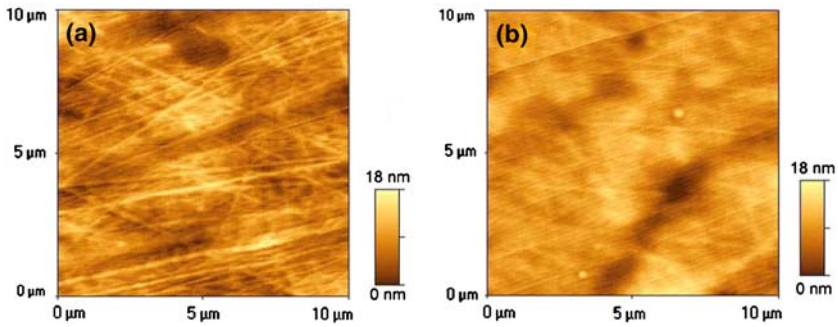

Fig. 6 AFM images (10 µm × 10 µm) of the PC surface of CDs before (**a**) and after UV/ozone treatment for 10 min (**b**)

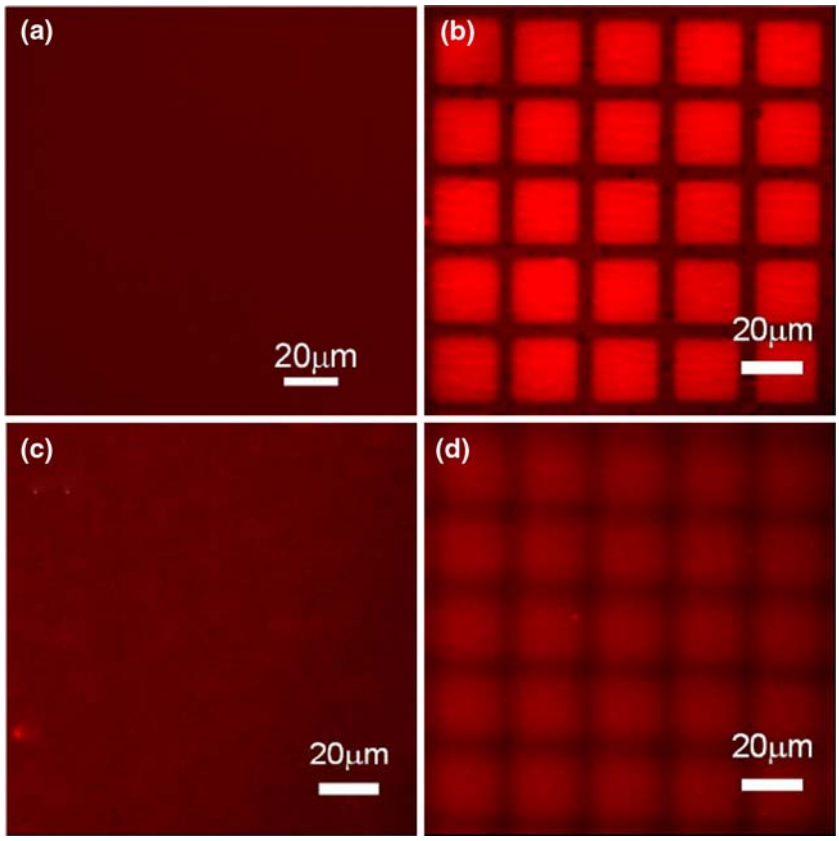

Fig. 7 Fluorescent images of PC surfaces of CDs: (**a**) upon the immobilization of amine-terminated DNA probes; (**b**) after hybridization with complementary fluorescein-labeled DNA targets; (**c**) the control experiment by first immobilizing non-complementary probe strands then hybridize with the same target as in (b); (**d**) upon the immobilization of single-base mismatch probe strands then hybridization with the same target strand in (b)

bases of CDs. The surface chemistry (activation, patterning, and coupling) involved is simple and efficient; and the hybridization is highly sensitive and selective. This work lays the foundations for the development of disposable plastic biochips (not limited to DNA microarrays) and of biomedical devices that are readable by conventional CD drives.

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