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# Light-Directed Synthesis of High-Density Peptide Nucleic Acid Microarrays

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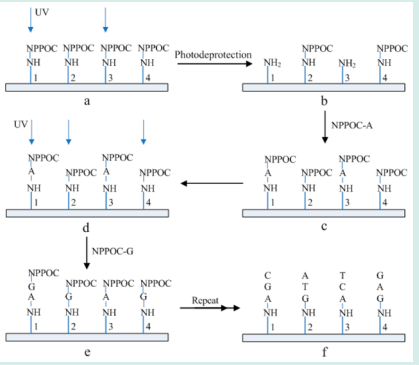
Supporting Information

**ABSTRACT:** Peptide nucleic acids (PNAs) are a class of nucleic acid mimics that can bind to the complementary DNA or RNA with high specificity and sensitivity. PNA-based microarrays have distinct characteristics and have improved performance in many aspects compared to DNA microarrays. A new set of PNA monomers has been synthesized and used as the building blocks for the preparation of high density PNA microarrays. These monomers have their backbones protected by the photolabile group 2-(2-nitrophenyl)propyloxy carbonyl (NPPOC), and their exocyclic amino groups protected by amide carbonyl groups. A light-directed synthesis system was designed and applied to the in situ synthesis of a PNA microarray with a density of over 10 000 probes per square centimeter. This PNA microarray was able to detect single and multiple base-mismatches correctly with a high discrimination ratio.




**KEYWORDS:** *peptide nucleic acid, NPPOC, monomer, microarray, photodeprotection*

## INTRODUCTION

Microarrays can provide an accurate, high-throughput parallel analysis in biochemistry and medical diagnosis.[1,2] In particular, nucleic acid microarrays have become a powerful tool for both gene related biomedical and clinical research.[3−7] Such microarray analysis is based on the specific hybridization between immobilized nucleic acid probes on a solid substrate and their complementary targets. However, the hybridization efficiency and accuracy of DNA-based microarray are greatly influenced by temperature, ionic strength, and the affinity between probes and targets.[8] Peptide nucleic acids (PNA), first synthesized by Nielsen et al. in 1991,[9] are a unique class of nucleic acid mimics in which the sugar phosphate backbone is replaced with a polyamide backbone composed of *N*-(2-aminoethyl)glycine units. PNA probes can recognize their complementary DNAs, RNAs, or PNAs, obeying Watson−Crick base-pairing rules with remarkably higher specificity and affinity than that of DNA probes.[10−12] The neutral backbone of the PNA can reduce electrostatic repulsion between the probe and the DNA target, and thereby facilitate hybridization.[13] PNAs have shown to retain their efficient hybridization properties in solution after attachment to the surfaces of substrates.[14−16] PNA probes can hybridize with DNA targets in buffers with low ionic strength, which can lower the possibility of forming DNA secondary or tertiary structures. Under physiological conditions, the PNA−DNA duplexes have higher melting temperatures than corresponding DNA−DNA duplexes by approximately 1.5 °C per base pair.[17] In addition, unlike DNA probes that can only detect single-stranded nucleic acid targets, PNA-based probes can bind to double-stranded DNA,[18−22] which makes it possible to achieve direct detection of clinical specimens. In fact, analysis of relatively small arrays of PNA oligomers have demonstrate their potential as excellent alternatives to DNA microarrays.[23−26] Similar to the synthesis of DNA microarrays, PNA microarrays can be prepared using spotting or in situ synthesis approaches.[27−29]

Liu et al.[30,31] successfully prepared photolabile o-nitroveratryloxycarbonyl (NVOC) protected PNA monomers, and then synthesized a PNA microarray using a maskless array synthesis system. However, the photodegradation products of the NVOC contain carbonyl compounds, which can react with amino groups and reduce stepwise synthetic yield. Moreover, the microarray contains only 256 spatially defined PNA probes that are not sufficient for high throughput analysis. Meanwhile, the high cost of PNA monomers has become one of major barriers to the application of PNA microarray.[32] In this Research Article, a set of more photolabile PNA monomers, with their exocyclic amino groups protected by base labile amide carbonyl groups, was developed, and high density PNA microarray was synthesized using 2-(2-nitrophenyl)-propyloxycarbonyl (NPPOC) protected PNA monomers through light-directed in situ synthesis.

## EXPERIMENTAL PROCEDURES

**Synthesis of PNA Monomers.** The chemical synthesis of PNA monomers relies on the assembly of protected *N*-(2-aminoethyl)glycine backbone and protected nucleobase-substituted acetic acid structural units. We chose NPPOC as the

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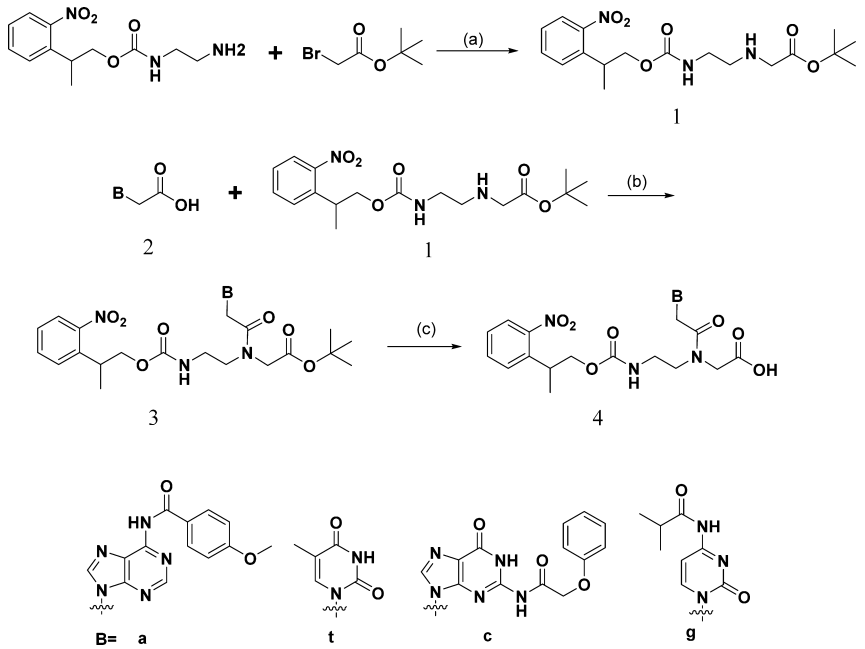
**Scheme 1**[a]




[a]Reagents and conditions: (a) $CH_2Cl_2$ ;(b) DMF, *N,N*-diisopropylethylamine, BOP, HOBT, ethyl acetate/$H_2O$; (c) dichloromethane, triethylsilane, trifluoracetic acid.

photolabile protecting group since it has been successfully used in the synthesis of DNA and peptide microarrays.[33−37] NPPOC group can be removed by irradiation at wavelengths >300 nm without damaging the nucleic acids or the backbone. Compared to NVOC group, NPPOC group has a higher quantum efficiency.[38] NPPOC group can be removed effectively in about 2 min (365 nm, 15 mW/cm$^2$), which is much shorter than that of the NVOC group (20 min, 365 nm, 15 mW/cm$^2$).[31] Therefore, the time needed for the synthesis of PNA microarray can be shortened considerably.

The synthesis of PNA monomers was carried out according to the route list in Scheme 1. The key intermediate in the synthetic route to the PNA building blocks is *tert*-butyl *N*-[2-(2-nitrophenyl)propyloxy carbonyl)aminoethyl] glycinate (**3**), which was synthesized according to the method reported in the literature.[39] The exocyclic amino groups of adenine, cytosine, and guanine were blocked with base sensitive acyl methoxybenzoyl(An), isobutyryl (*i*Bu), and phenoxyacetyl (Pac) groups, respectively.[40−42] Protected adenine, cytosine, and guanine were converted to their sodium salt using NaH in DMF and alkylated using butyl bromoacetate. The resulting butyl ester was saponified using trifluoracetic acid in dichloromethane.[39] 2-(3,4-Dihydro-5-methyl-2,4-dioxopyrimidin-1(2*H*)-yl)acetic acid (2t) was acquired by substituting the bromide molecular of bromoacetic acid with thymine.[43] The carboxymethylated nucleobases were coupled to the backbone *tert*-butyl *N*-[2-(2-nitrophenyl)propyloxy carbonyl)aminoethyl] glycinate (**3**) using standard methods, which were followed by a removal of the *tert*-butyl ester under standard conditions,[30,31] as shown in Scheme 1. The final PNA monomers associated with adenine, cytosine, and guanine have their exocyclic amino groups blocked with methoxybenzoyl, isobutyryl, and phenoxyacetyl, respectively. The four monomers are abbreviated as NPPOC-A, NPPOC-C, NPPOC-G, and NPPOC-T in the following text.

*N-[2-(2-Nitrophenyl)propyloxycarbonyl)aminoethyl]-N-{[6-N-(4-methoxybenzoyl) adenin-9-yl]acetyl}glycine (NPPOC-A)* ***4a***. Compound **3a** (5.837 g, 8.45 mmol) was dissolved in MC (30 mL), then triethylsilane (4 mL) and TFA (30 mL) were added to the solution. The reaction mixture was stirred at room temperature for 4 h. TFA was removed by coevaporation under vacuum with petroleum ether and MC. Purification by chromatography gave product **4a** with a yield of 4.156 g (77.5%). MS + 1, *m*/*z* 635.1 ($C_{29}H_{30}N_8O_9$ + $H^+$, theoretical 635.2). $^1$HNMR (500 M Hz, DMSO-$d_6$): $\delta$ = 0.85 (dt, *J* = 20, 7 Hz, 2H), 1.27 (d, *J* = 10 Hz, 3H), 3.04 (q, *J* = 6.5 Hz, 1H), 3.29−3.31 (m, 2H), 3.87 (s, 3H), 3.97 (s, 1H), 4.09−4.15 (m, 1H), 4.16−4.25 (m, 2H), 5.17 and 5.33 (2s, 2H), 7.09 (d, *J* = 10 Hz, 2H), 7.22 (dt, *J* = 80, 5.5 Hz, 1H), 7.43−7.49 (m, 1H), 7.65−7.68 (m, 1H), 7.69−7.70 (m, 1H), 7.81 (t, *J* = 10 Hz, 1H), 8.06 (dd, *J* = 4 Hz, 2 Hz, 2H), 8.31 (d, *J* = 2 Hz, 1H), 8.67 (d, *J* = 5 Hz, 1H), 11.01 (d, *J* = 4 Hz, 1H), 12.90 (s, 1H).

*N-[2-(2-Nitrophenyl)propyloxycarbonyl)aminoethyl]-N-[[4-N-(4- isobutyryl)-cytosin-1-yl]acetyl]glycine (NPPOC-C)* ***4c***. Compound **4c** was prepared, purified, and characterized in the same way as described for **4a**: yield 74.7%; MS + 1, *m*/*z* 547.1 ($C_{24}H_{30}N_6O_9$ + $H^+$, theoretical 547.2). $^1$HNMR (500 M Hz, DMSO-$d_6$): $\delta$ = 0.82−0.87 (m, 2H), 1.07 (d, *J* = 6.5 Hz, 6H), 1.26 (d, *J* = 6.5 Hz, 3H), 3.05 (q, *J* = 6.5 Hz, 1H), 3.20−3.24 (m, 2H), 3.96 (s, 1H), 4.09−4.16 (m, 2H), 4.18−4.22 (m, 1H), 4.62 and 4.80 (2s, 2H), 7.19−7.22 (m, 2H), 7.45−7.48 (m, 1H), 7.66−7.69 (m, 1H), 7.69−7.71 (m, 1H), 7.82 (d, *J* = 7.5 Hz, 1H), 7.90 (dd, *J* = 8, 7.5 Hz, 1H), 10.84 (d, *J* = 13.5 Hz, 1H), 12.71 (s, 1H).

*N-[2-(2-Nitrophenyl)propyloxycarbonyl)aminoethyl]-N-[[2-N-(phenoxyacetyl)guanin-9-yl]acetyl]glycine (NPPOC-G)* ***4g***. Compound **4g** was prepared, purified, and characterized in the same way as described for **4a**: yield 80.85%; MS + 1, *m*/*z* 651.2 ($C_{29}H_{30}N_8O_{10}$ + $H^+$, theoretical 651.2). $^1$HNMR (500 M Hz, DMSO-$d_6$): $\delta$ = 0.85 (dt, *J* = 20, 7 Hz, 2H), 1.23−1.26 (m, 3H), 3.07 (q, *J* = 6.5 Hz, 1H), 3.41−3.47 (m, 2H), 3.89 (s,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1H), 4.07−4.17 (m, 2H), 4.19−4.23 (m, 1H), 4.84 (d, $J$ = 5 Hz, 2H), 4.93 and 5.10 (2s, 2H), 6.95−6.96 (m, 2H), 6.97−6.99 (m, 1H), 7.29−7.32 (t, $J$ = 8 Hz, 2H), 7.33−7.37 (m, 1H), 7.44−7.48 (m, 1H), 7.67 (d, $J$ = 5 Hz, 1H), 7.69−7.70 (m, 1H), 7.80−7.81 (m, 1H), 7.83 (q, $J$ = 4 Hz, 1H), 11.85 (s, 1H).

*N-[2-(2-Nitrophenyl)propyloxycarbonyl)aminoethyl]-N-[(thymin-l-yl)acetyl]glycine (NPPOC-T)* ***4t***. Compound **4t** was prepared, purified, and characterized in the same way as described for **4a**: yield 88.5%; MS + 1, $m/z$ 491.9 ($C_{21}H_{25}N_5O_9$ + $H^+$, theoretical 492.0). $^1$HNMR (500 M Hz, DMSO-$d_6$): $\delta$ = 0.85 (dt, $J$ = 20, 7 Hz, 2H), 1.26 (d, $J$ = 10 Hz, 3H), 1.75 (s, 3H), 3.04 (q, $J$ = 6.5 Hz, 1H), 3.17−3.21 (m, 2H), 3.95 (s, 1H), 4.12−4.16 (m, 2H), 4.18−4.21 (m, 1H), 4.45 and 4.61 (2s, 2H), 7.12 (dt, $J$ = 70, 5.5 Hz, 1H), 7.28 (d, $J$ = 30 Hz, 1H), 7.45−7.49 (m, 1H), 7.66−7.68 (m, 1H), 7.69−7.70 (m, 1H), 7.81 (d, $J$ = 8 Hz, 1H), 11.29 (d, $J$ = 6 Hz, 1H), 12.76 (s, 1H).

**Light Projection and Reaction System.** The light-directed PNA microarray synthesis system developed in the lab is composed of a light projection system and a reaction chamber.[44] The projection system included a UV light source, a light modulation system, a digital micromirror device (DMD, Texas Instruments), and a reaction chamber,[45] as shown in Figure 1. The light source is a high-pressure mercury lamp

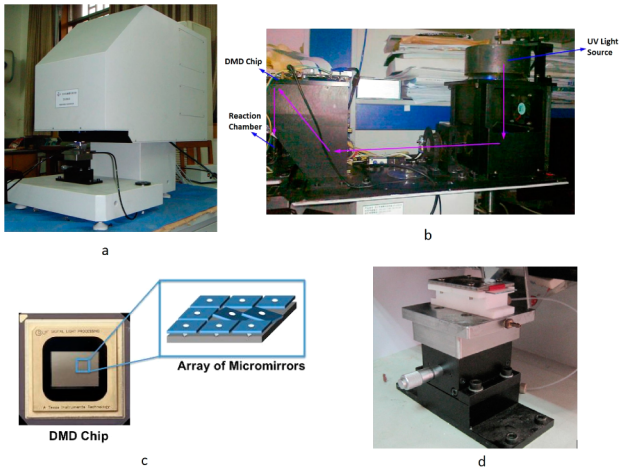


**Figure 1.** Light projection and reaction system: (a) light projection system, (b) light path in the light projection system, (c) digital micromirror device (DMD, Texas Instruments), and (d) reaction chamber.

made by OSRAM (BHO350W/S). The DMD used in the system (DLP7000UV) has 1024 × 768 mirror pixels with each micromirror having a 13.6 × 13.6 $\mu$m reflection area. The combination of the states of mirrors forms a pattern of selectively distributed light spots on the surface of the reaction substrate. After modulating, the light spots projected onto the glass substrate have the same size as the mirror pixels. As a result, this system has the maximum capacity of synthesizing 786 432 different PNA oligmer spots with each spot having a dimensions of 13.6 × 13.6 $\mu$m. In this paper, combinations of mirrors were used to form elements in the patterns.

**Glass Surface Modification.** The following procedure was used successively to treat glass surfaces to obtain NPPOC-functionalized glass slides.

*Amination.* The glass slides were treated in the mixture of $H_2SO_4$ and $H_2O_2$ (4:1, v/v) for 30 min, rinsed with deionized water, acetone, and then dried under vacuum. The glass slides were silanized in the solution of 5% (v/v) GPTS in chloroform at 50 °C for 12 h. Sonication in chloroform was performed for 10 min to remove the noncovalently attached GPTS molecules, then the glass substrates were rinsed with acetone, and blown dry with nitrogen. The slides were immersed in the solution of 5% (v/v) 4,7,10-trioxa-1,13-tridecanediamine in chloroform at 50 °C for 12 h, after which the slides were sonicated in chloroform for 10 min, then rinsed extensively with acetone, and finally blown dry with nitrogen.

*Attachment of Spacer and NPPOC Group.* Using Fmoc-chemistry, the molecules on the glass surface were extended with a peptide spacer 6-aminocaproyl-6-aminocaproic acid. This extension can facilitate the interaction between the target sequence and the PNA probe. Specifically, the aminated glass slides were immersed in the solution of Fmoc-ACA (5 mM), BOP (5 mM), HOBt (5 mM), and DIEA (10 mM) in DMF at 25 °C for 2 h. The slides were then rinsed with DMF, and the Fmoc-groups were removed by incubating them in a solution of 20% piperidine in DMF for 5 min. After two synthesis cycles of Fmoc-chemistry occurred, the slides were washed with DMF, then rinsed with MC, and finally dried with nitrogen stream. 2-(2-Nitrophenyl)propyloxycarbonyl aminocaproic acid (NPPOC-ACA)[46,47] was attached to the molecules on the glass surface to produce a layer of NPPOC groups.

**Light-Directed PNA Microarray Synthesis.** The process of light-directed synthesis of PNA microarrays is outlined in Figure 2. Initially, the amino groups on the glass substrate are

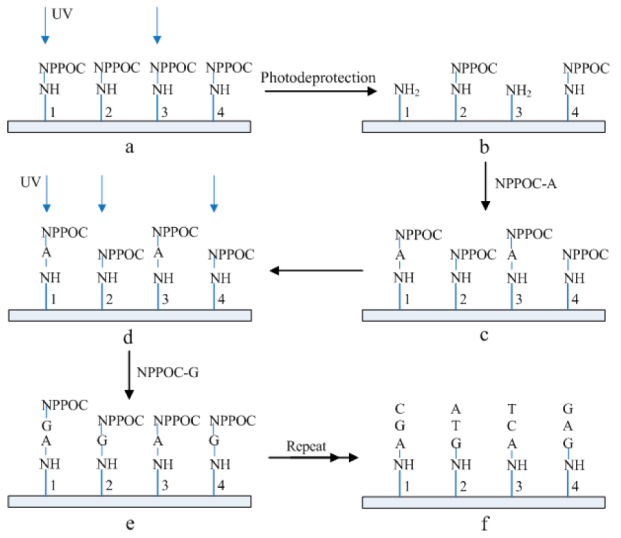


**Figure 2.** Process of the synthesis of PNA microarrays via photolithographic technique. (a) Ultraviolet illumination of spots 1 and 3. (b) Photolabile protecting group (NPPOC) removed at spots 1 and 3 to reveal amino groups. (c) Coupling of NPPOC-A to the amino groups at spots 1 and 3. (d) Ultraviolet illumination to remove NPPOC groups at spots 1, 2, and 4. (e) Coupling of NPPOC-G to the amino groups exposed at spots 1, 2, and 4. (f) PNA array obtained after several additional synthesis cycles.

blocked by the NPPOC protecting group. Illumination of specific regions leads to photodeprotection. Amino groups in the exposed spots of the substrate are now accessible for coupling. The first monomer (e.g., NPPOC-A) is then coupled with the exposed amino groups. A different pattern of illumination is used to photoactivate a different region of the substrate. The second monomer (e.g., NPPOC-G) is coupled with the newly exposed amino groups. Additional cycles of


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photodeprotection and of coupling are carried out to obtain the desired PNA array. Finally, the surface is uniformly illuminated to remove all NPPOC groups on the spots.

The photolysis steps were performed in the presence of deionized water. Specific regions of the surface were irradiated through a DMD controlled projection, as described above, for 2 min using the 365 nm UV light with an intensity of 15 mW/cm$^2$. The reaction chamber was washed with deionized water and ethanol, subsequently, and then dried with nitrogen stream prior to the coupling reaction. In the coupling reaction, a 0.04 M solution of the HATU-activated PNA monomer in *N*-methylpyrrolidone (NMP) was prepared by mixing equal volumes of the following: NPPOC-protectd PNA monomer (0.18 M), the base solution (0.18 M of DIEA and 0.27 M of 2.6-lutidine) and HATU (0.162 M). After the preactivation for 2 min, the PNA monomer solution was introduced into the reaction chamber, and then allowed to couple with exposed amino groups for 20 min at room temperature. The reaction chamber was then washed with DMF and ethanol. After coupling, a solution of 5% acetic anhydride and 6% 2,6-lutidine in DMF was introduced into the reaction chamber to cap the free, nonelongated, amino groups for 5 min. This capping method was used in a previous study, which demonstrated to be efficient.[31] Then, the reaction chamber was washed extensively with DMF and ethanol and dried with nitrogen stream. Deprotection, coupling, and capping were repeated as described above until the synthesis of the PNA microarray was completed. After the coupling of the last monomer, the terminal NPPOC group on each PNA probe was removed by a final photolysis reaction that covers all reaction spots. The glass slide was washed extensively and dried. Side-chain protecting groups were removed by treating the microarray with ethanolamine/ethanol (1/1, v/v) for 2 h at 75 °C.

## RESULTS AND DISCUSSION

**Photolysis of the NPPOC Group.** To determine the optimum procedure for the photodeprotection of NPPOC group, a series of exposure experiments were carried out using NPPOC-ACA modified glass substrates. In the experiments, light-directed PNA microarray synthesis system was used to pattern the modified glass slide with different periods of UV illumination time in different solvent environments. To visualize the pattern of the deprotected amino group, the slides were treated with 1 mM RBITC solution in NMP, resulting in the coupling of a fluorophore onto the free amino groups, which can be imaged with a fluorescence scanner.

Under UV illumination, the NPPOC group is cleaved under a $\beta$-elimination process with the formation of *o*-nitrostyrene, $CO_2$ along with the corresponding amino compound.[48,49] Walbert et al.[50] reported that with an variation of the percentage of $H_2O$ in a MeCN/$H_2O$ solvent mixture, the yield of styrene ($\beta$-elimination pathway) and nitroso product are complementary. $\beta$-Elimination is favored under the conditions where the deprotonation of the aci-nitro compound is efficient. With an increase of acidity in MeCN/$H_2O$ (1:1, v/v), the $\beta$-elimination pathway is gradually suppressed. This result is consistent with the experiments conducted by Beier et al.,[51] who proposed that the presence of aprotic basic solutions during irradiation could enhance photoremoval of NPPOC moieties. For example, the efficiency of photoremoval was remarkably higher with 0.05 M DBU in acetonitrile compared to dry, methanolic, or acetonitrile conditions. Our experiments showed that the presence of DBU during photodeprotection can lead to the cleavage of spacer on the modified glass slides and, hence, result in decreased signal intensities. After comparison, we chose a deionized water environment for the UV illumination. The photolysis of NPPOC group in the presence of water was completed in 2 min (15 mW/cm$^2$, 365 nm), as shown in Figure 3. In a prolonged illumination in water, the fluorescence intensity showed a slow decrease, but this decrease was not significant in the time frame for synthesizing 17-mers PNA probes.

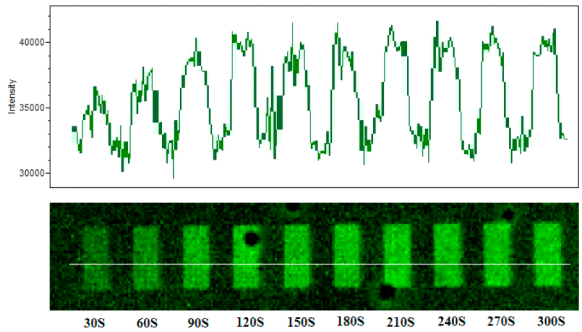


**Figure 3.** Fluorescence image of the deprotection of NPPOC group. Complete deprotection was achieved after 120 s of UV illumination. The fluorescence image was acquired from Genepix Pro 4000B Scanner (Packard, Billerica, MA, USA).

**Coupling Efficiency Analysis.** Quality of the probes strongly depends on the stepwise coupling efficiency of the monomers in the synthesis of PNA microarray. Stepwise coupling efficiency was determined as outlined in Figure 4.

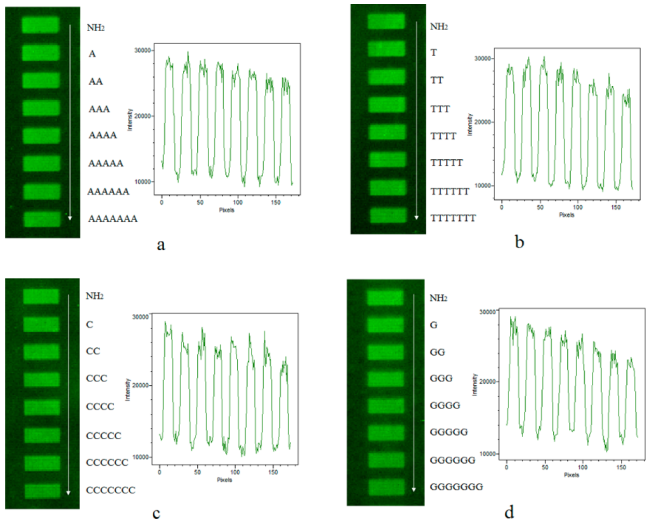


**Figure 4.** Coupling efficiencies of the PNA monomers. Fluorescence images of PNA oligomers with different lengths of monomer A (a), T (b), C (c), and G (d). The coupling efficiencies for seven steps were measured to be 88.2%, 85.3%, 86.7%, and 84.5%, and the average stepwise yields were calculated to be 98.22%, 97.75%, 97.98%, and 97.62% for A, T, C, and G, respectively.

Oligomers with different lengths were synthesized on the substrate according to the procedures described earlier. After first layer of T was coupled to the substrate, the spots corresponding to the second through seventh layers were irradiated, after which the second layer was coupled, and so on. Capping of the unreacted amino groups was carried out after



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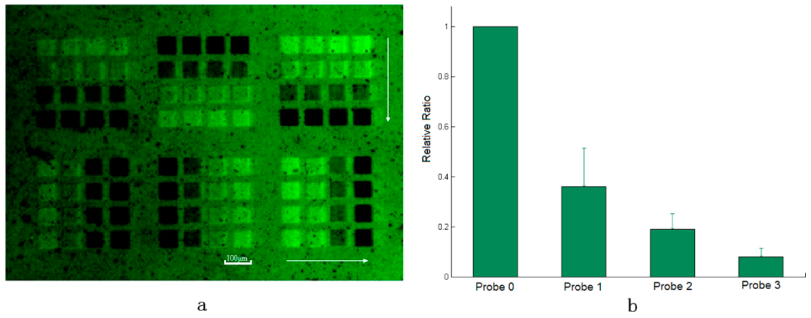


**Figure 5.** Hybridization of the PNA microarray to DNA target sequence. (a) Hybridization fluorescence pattern of DNA probe 5′-GCC TGA TTC AGG TAA CG-3′-TAMRA to PNA microarray containing four different 17-mers probes (probes 0, 1, 2, and 3 in the direction of the arrows). Hybridize spots are square shaped with a side length of 72 $\mu$m. (b) Plot of the average fluorescence intensity of the four parallel probes hybridized with the target. The average fluorescence intensity ratio of sequences with 0 to 3 mismatches was 1:0.36:0.19:0.08.

each step of coupling reaction. Finally, all of the spots were irradiated to remove NPPOC groups. The slide was then treated with 1 mM RBITC solution in NMP to visualize the exposed amino groups.

It is clear from Figure 4 that the fluorescence intensities of PNA oligomers with different lengths of A, T, C, and G decrease with the increase of oligomer length. The ratio of fluorescence intensities between the two sites provides a measure of the coupling efficiency. The coupling efficiencies for seven steps were measured to be 88.2%, 85.3%, 86.7%, and 84.5%, and the average stepwise yields were calculated according to the formula $(X)^{7/2}$ (where $X$ represents the total coupling efficiencies) to be 98.22%, 97.75%, 97.98%, and 97.62% for A, T, C, and G, respectively, which are higher than the coupling efficiency of NVOC protected PNA monomers[30,31] and consistent with a similar experiment carried out by Bhushan.[39] The coupling efficiency satisfies the requirement for the PNA microarray synthesis.

**PNA Microarray Hybridization.** To investigate the validity of the PNA array produced by in situ synthesis on the glass surface through photolithography. The test probe 0 with the sequence 3′-CGG ACT AAG TCC ATT GC-5′-NH2, which is a perfect match to the target was designed (the corresponding DNA target 5′-GCC TGA TTC AGG TAA CG-3′-TAMRA). This test sequence contains all possible coupling combinations, with nearly the same composition of the four monomers, and thus can be considered as a good model probe for the evaluation of perfect match and mismatch hybridization. For the detection of mutations, probe 1, 3′-CGG ATT AAG TCC ATT GC-5′-NH2, with a G:T mismatch pair was designed for single base mismatch detection. The other two probes, probe 2, 3′-CGG ATT AAC TCC ATT GC-5′-NH2 and probe 3, 3′-CGG ATT AAC TCC GTT GC-5′-NH2, with 2 and 3 mismatches, respectively, were also designed in the synthesized PNA microarray for the testing of multiple bases mismatches.

The obtained PNA array was hybridized with a TAMRA labeled DNA target in 0.1× S Sarc buffer (60 mM sodium chloride, 6 mM sodium citrate, 0.72% (v/v) *N*-lauroylsarcosine sodium salt solution) at 55 °C for 2 h. Hybridized array was then scanned with Genepix Pro 4000B Scanner. The results of the hybridization are shown in Figure 5. A significant difference was observed between the matched and mismatched sequences. The hybridized spots in Figure 5a are square shaped with a side length of 72 $\mu$m. The bright spots contained the probe 0 sequence, and the three faint spots, in the direction of the arrows, contained the probes 1, 2, and 3 sequences, respectively. Figure 5b shows the plot of the average fluorescence intensity of the four hybridized spots. The fluorescence intensity was not uniform across the microarray because of nonuniform reagents flow in the reaction chamber. For each set of perfect match and mismatch sequences, the intensity of the perfect match was set as 1, and the relative intensity of the mismatches were calculated based on the intensity of the perfect match. The average intensity ratio of sequences with 0 to 3 mismatches was 1:0.36:0.19:0.08. This result indicates that PNA array fabricated by the light-directed PNA microarray synthesis system can efficiently screen single and multiple base mismatches.

In Figure 5a, the total hybridization signal is not strong, also the signal intensity of the spots corresponding to probe 2 and 3 are very low (darker than background). Similar phenomenon was observed in other literatures.[28,52] This is most likely due to the damage to the spacer or substrate surface by illumination or postsynthesis treatment, which leads to the loss of probes. Phillips et al.[53] studied the in situ oligonucleotide synthesis on carbon materials, and implied that the intrinsic hydrolytic instability of the siloxyl linkage employed in glass chemical modification can dramatically affect the stability of the synthesized oligonucleotides. In spite of the lost signal at the spots, the microarray provides good discrimination between matched and mismatched sequences.

## CONCLUSIONS

Four PNA monomers were successfully synthesized and then used as building blocks for the preparation of high density PNA microarray. The hybridization result showed that this microarray can effectively discriminate perfect matched and mismatched sequences. Each probe spot has the shape of a square with a side length of 72 $\mu$m, which means that the density of more than 10,000 probes per square centimeter can be achieved on a substrate. The limiting factor of the spot size in our experiment is not the light-directed PNA microarray synthesis system or the NPPOC chemistry, but the available scanner which had a 5 $\mu$m scan resolution. By having the access to more elaborate scanners, the limiting factor will switch to the size of DMD pixels. Consequently, the density of the microarray can be increased. Meanwhile, compared with previous reports, only half reaction time was required for each synthesis cycle without compromising the coupling efficiency.


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## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscombsci.5b00074.

Chemicals and instruments used in this study and NMR spectra of PNA monomers (PDF)

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### Notes

The authors declare no competing financial interest.

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