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Spatial Photorelease of Oligonucleotides, Using a Safety-Catch Photolabile Linker

Shane T. Flickinger,[†,#] Madhusudan Patel,[†,§,#] Brock F. Binkowski,[‡] Aaron M. Lowe,[⊥,§] Mo-Huang Li,[¶,§] Changhan Kim,[¶,§] Franco Cerrina,[¶,§] and Peter J. Belshaw*[,†,‡]

The Department of Chemistry, The Department of Biochemistry, The Department of Computer and Electrical Engineering, Materials Science Program, and Center for Nanotechnology, University of Wisconsin−Madison, Madison, Wisconsin 53706

belshaw@chem.wisc.edu

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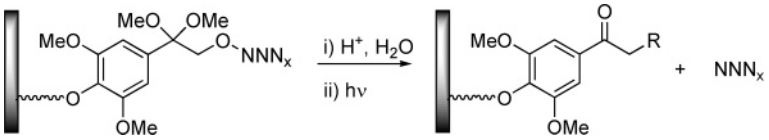
ABSTRACT



We report the development of a safety-catch photolabile linker that allows the light-directed synthesis and spatially selective photorelease of oligonucleotides from microarrays. The linker remains stable to light during DNA synthesis, and is activated for photorelease after acidic hydrolysis. We demonstrate that the photoreleased oligonucleotides can be amplified by PCR to produce double stranded DNA. The advantages offered by this linker could aid the development of an automated gene synthesis platform.

The in situ, light-directed synthesis of oligonucleotides in microarrays, first introduced by Fodor and co-workers at Affymetrix,[1] has become a powerful diagnostic tool. In this approach, oligonucleotide microarrays are synthesized through iterative deprotection and coupling cycles, using nucleoside phosphoramidites that can be deprotected directly or indirectly with light.[1,2] More recently, the photodeprotections have been mediated by a maskless array synthesizer (MAS).[3] The MAS uses light reflected from a digital micromirror device, enabling the production of oligonucleotide arrays with up to 786 432 features. This approach allows for the rapid and flexible synthesis of an oligonucleotide microarray with any desired set of sequences, inviting the prospect of utilizing oligonucleotides synthesized in microarrays as templates for gene assembly (Figure 1, Supporting Information). Indeed, several methods for gene assembly from microarrays have already been reported.[4]

A method to selectively release oligonucleotides in a controlled, spatial manner from subregions of a microarray

[†] Department of Chemistry.
[‡] Department of Biochemistry.
[§] Center for Nanotechnology.
[⊥] Materials Science Program.
[¶] Department of Computer and Electrical Engineering.
[#] These authors contributed equally to this work.

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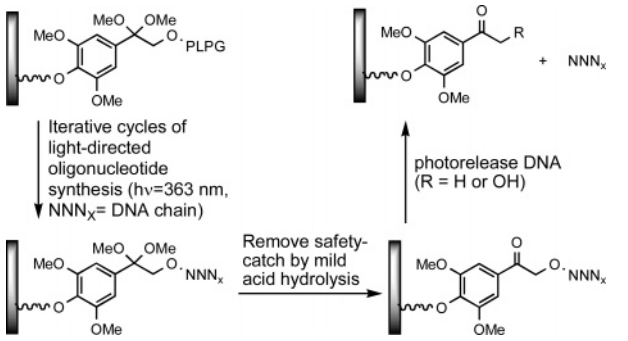

Figure 1. Use of a safety-catch photolabile linker for light-directed synthesis and release of oligonucleotides.

could aid the development of a robust gene assembly platform. Chemical methods of elution, such as the basic hydrolytic cleavage of linkers,[5] have the disadvantage of releasing oligonucleotides from all exposed areas of the array surface. Selective release of oligonucleotides from the array surface could be accomplished through a microfluidic approach, such that a cleavage reagent is delivered only to specific regions of the array. Here we report a safety-catch photolabile linker (SC-PLL) that allows for both light-directed synthesis and spatially selective photorelease of oligonucleotides in microarrays.

A review of photolabile protecting groups (PLPGs) and photolabile linkers (PLLs)[6] presented two potential strategies for linker design: (1) wavelength selective photolysis or (2) latent photocleavable linkers. The independent, orthogonal deprotection of PLPGs has been achieved through the use of compounds that respond selectively to light of differing wavelengths.[7] However, the reported selectivity is not absolute and is limited by the broad absorption spectra of these photolabile protecting groups. As the synthesis of an oligonucleotide microarray requires many irradiation cycles prior to release, wavelength selective release was not deemed practical. SC-PLL's based on the benzoin PLPG have previously been developed to reduce light-sensitivity and improve the chemical stability of PLPGs used in syntheses.[8]

A SC-PLL for oligonucleotide synthesis and release must be compatible with the conditions of DNA synthesis, and activation/photolysis conditions must be compatible with DNA. We chose to develop our safety-catch based on the phenacyl PLPG[9] since masking the carbonyl as a dimethyl ketal renders this group photoinert during oligonucleotide synthesis. Postsynthesis activation of the safety-catch by mild acidic hydrolysis enables selective photorelease (Figure 1).

Synthesis of the SC-PLL (Scheme 1) began with 3′,5′-dimethoxy-4′-hydroxyacetophenone **1** and ethylene glycol. Ethylene glycol was monoprotected with TBS-Cl, and then coupled to **1** via Mitsunobu reaction to yield **2** (87%). Treatment of **2** with $PhI(OAc)_2$ in basic MeOH gave **3** (84%).[10] The free alcohol was protected with NPPoc-Cl[11] and deprotected with TBAF to give **4** (89%). Finally, treatment with 2-cyanoethyl diisopropylchlorophosphoramidite gave phosphitylated linker **5** (86%), ready for attachment to functionalized surfaces.[12]

To investigate the conditions for activation and photorelease of SC-PLL **5**, monohydroxysilane derivatized glass slides[3a] were prepared and extended with two NPPoc-hexaethylene glycol-phosphoramidite spacers (analogous to previously reported MeNPoc-hexaethyleneglycol-phosphoramidites),[11c] followed by coupling of the SC-PLL **5**. Intermediate phosphites were oxidized to the phosphotriesters with I_2/H_2O/pyridine solution. A series of square features containing T_{20}-oligomers were synthesized via standard protocols,[13] using iterative cycles of photodeprotection and coupling/oxidation of NPPoc-protected nucleoside phosphoramidites (Proligo), followed by deprotection with a 1:1 solution of ethylenediamine and ethanol. Small squares inside the larger square features were irradiated for varying times either before or after hydrolysis of the dimethyl ketal with acid. For acid activation of the linker, 3% trichloroacetic acid (w/v) in 95:5 acetone/MeOH was used to minimize nonspecific stripping from the glass slides and potential depurination of oligonucleotides, while allowing rapid activation of the linker. Thioxanthone or 1-chloro-4-propoxy-9*H*-thioxanthone (CPT) was used to enable the efficient, long wavelength (>360 nm) photolysis of the phenacyl group. The exact mechanism of thioxanthone mediated photorelease remains undetermined; however, it may function as a photoreductant or as a triplet sensitizer.[14] The slide was visualized by hybridization to a complementary Cy3-labeled A_{29} probe (Figure 2a). Selective and spatially controlled photorelease of oligonucleotides was revealed by loss of fluorescence in small inner squares only after acid activation of the safety-catch. No photorelease of the oligomer was observed prior to acid activation.

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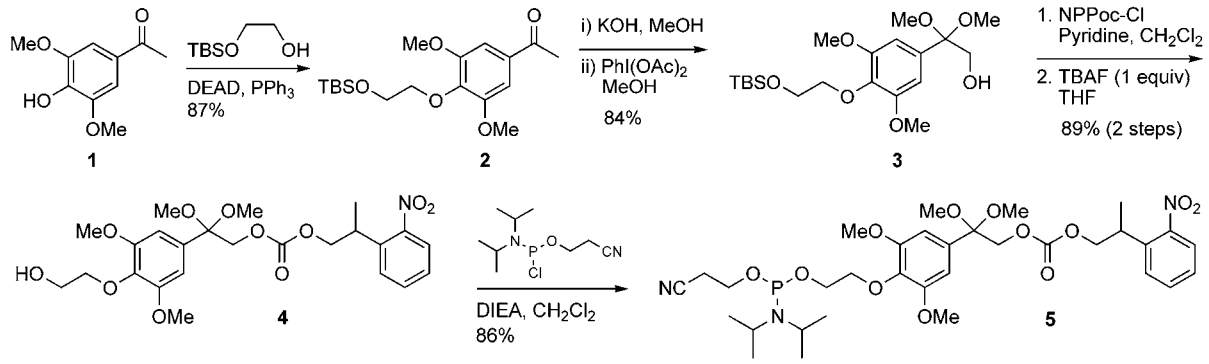
Scheme 1. Synthesis of the SC-PLL



To more directly demonstrate spatial photorelease and assess the integrity of the released oligonucleotides we prepared an array with four features containing T_{20} and T_{28} oligomers (Figure 4, Supporting Information). After acid activation, approximately half of each oligonucleotide region was photoreleased and collected separately. The T_{28} oligomer was photoreleased first followed by release of the T_{20} oligomer. The remaining oligonucleotides on the slide were chemically released with concentrated ammonium hydroxide.[15] The oligonucleotides were radiolabeled with ^{32}P ATP and analyzed by polyacrylamide gel electrophoresis. The gel shows the spatially selective release of the T_{28} (Figure 2b, lane 1) and T_{20} oligomers (Figure 2b, lane 2). The chemical release (Figure 2b, lane 3) shows the expected indiscriminate release of both the T_{28} and T_{20} oligomers. The gel profiles also show that the major species released from the slide are the desired T_{28} and T_{20} oligomers. These experiments demonstrate that the SC-PLL enables the spatial synthesis and spatial release of oligonucleotides in microarrays.

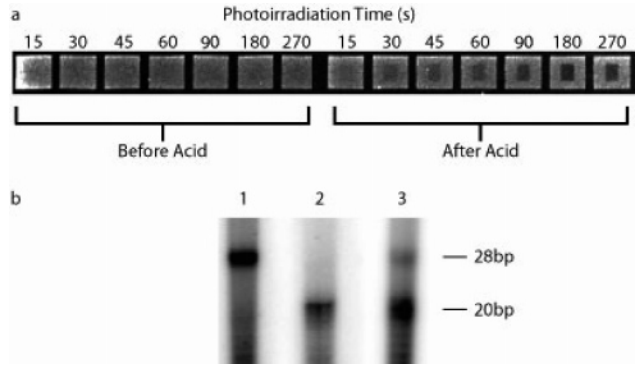


Figure 2. (a) Fluorescence scan of a series of T_{20} oligomer features (large squares, 640 μm^2) revealing selective, spatial photorelease of the T_{20} oligomers (small squares, 280 μm^2) after acid treatment as visualized by hybridization to a complementary Cy3-labeled A_{29} probe. (b) Denaturing electrophoresis gel profile of 5'-^{32}P-labeled T_{20} and T_{28} oligomers synthesized and photoreleased from the DNA microarray showing selective synthesis and photorelease of oligonucleotides. The SC-PLL was activated with 3% trichloroacetic acid (w/v) in 95:5 acetone/MeOH (20 min) and photoreleased in the presence of 0.01% CPT (270 s). Lane 1, photoreleased T_{28}; lane 2, photoreleased T_{20}; and lane 3, chemical release of T_{20} and T_{28} oligomers from the microarray (concentrated NH_4OH, 4 h, rt).

To investigate whether the photoreleased oligonucleotides are functional as templates for amplification, we synthesized and photoreleased a mixed base 40-mer that served as a template for PCR amplification. Figure 3 shows the PCR products analyzed by acrylamide gel electrophoresis. A major band of 40 bp in length appears only in the PCR reaction containing primers and the photoreleased template. The successful demonstration of PCR amplification of the photoreleased oligonucleotides should enable studies toward multiplexed gene assembly from a single microarray.[16]

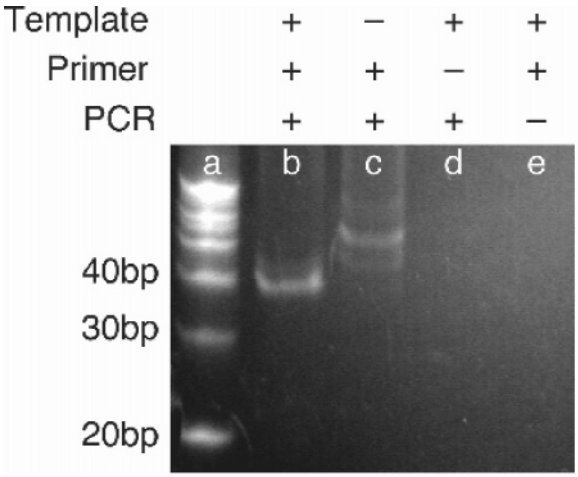


Figure 3. Acrylamide gel profile of a mixed base 40mer photoreleased from a microarray and amplified by PCR. Lane a, oligonucleotide markers (Amersham Biosciences); lane b, photoreleased 40mer amplified by PCR; lane c, water control (no template); lane d, template (no primer); and lane e, photoreleased 40mer (no PCR). Sequence of 40mer: 5'-TGC-CGG-AGT-CAG-CGT-AGG-ATA-TCT-CGT-GGC-GAC-TCT-GAC-T-3'.

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The modular design of our SC-PLL (Figure 2, Supporting Information) should enable the linker to be tuned for particular applications. The acid sensitivity of the safety-catch can be tuned by varying the nature of the ketal; NPPoc could easily be replaced by another PLPG; and the method of surface attachment can be modified. Although we currently employ thioxanthones to enable long-wavelength photolysis (>360 nm), sensitizing groups have also been incorporated directly into PLPGs.[17] In addition, our SC-PLL is not limited to light-directed oligonucleotide synthesis. It should be possible to mask the PLL with a safety-catch that can be removed via basic, oxidative, or reductive methods,[18] extending its utility to other microarray synthesis formats.[4b,c,19]

There are several potential advantages of using SC-PLL **5** for gene assembly in a microarray format. First, the SC-PLL gives, in theory, complete flexibility with regard to which sequences are released from the microarray, possibly enabling multiplexed PCR assembly reactions on subsets of photoreleased oligonucleotides from a single microarray. Second, SC-photorelease could potentially improve the fidelity of oligonucleotides eluted from the array surface by limiting the release of error containing sequences formed at the boundaries of features, due to partial deprotections and stray light.[20] This could be accomplished by selectively releasing the internal region of a photopatterned feature without releasing oligonucleotides at the edges of the feature (Figure 3, Supporting Information). Similarly, with an incomplete photorelease (e.g., photoreleasing to 50% completion), the higher quality oligonucleotides should be selectively released from the regions with highest light intensity within each pixel. Finally, using a SC-PLL to release oligonucleotides from subregions of the array should significantly reduce the number of unique primer sets needed for PCR based amplification. In addition, for small assemblies, a SC-PLL could eliminate the need for primer sets altogether.

In summary, our experiments collectively demonstrate that SC-PLL **5** enables the spatially arrayed synthesis and spatially controlled photorelease of oligonucleotides in microarrays, and that the oligonucleotides released are functional as templates for PCR amplification. Additionally, using a SC-PLL may improve the fidelity of eluted oligonucleotides, and reduce the number of primer sets needed for subsequent assembly reactions. These unique features will aid the development of an automated gene synthesis platform. The assembly of spatially released oligonucleotides from the SC-PLL into short double stranded DNA fragments as well as the design and synthesis of a SC-PLL that functions at long wavelengths (>360 nm) without the use of a sensitizer are areas of ongoing research.

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Supporting Information Available: Figures, synthesis, and spectra of SC-PLL **5**, surface chemistry, and PCR. This material is available free of charge via the Internet at http://pubs.acs.org.

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