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Assembling Semiconductor Nanocomposites Using DNA Replication Technologies

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Abstract

Deoxyribonucleic acid (DNA) molecules represent Nature's genetic database, encoding the information necessary for all cellular processes. From a materials engineering perspective, DNA represents a nanoscale scaffold with highly refined structure, stability across a wide range of environmental conditions, and the ability to interact with a range of biomolecules. The ability to mass-manufacture functionalized DNA strands with Ångstrom-level resolution through DNA replication technology, however, has not been explored. The long-term goal of the work presented in this report is focused on exploiting DNA and *in vitro* DNA replication processes to mass-manufacture nanocomposite materials. The specific objectives of this project were to: (1) develop methods for replicating DNA strands that incorporate nucleotides with "chemical handles," and (2) demonstrate attachment of nanocrystal quantum dots (nQDs) to functionalized DNA strands. Polymerase chain reaction (PCR) and primer extension methodologies were used to successfully synthesize amine-, thiol-, and biotin-functionalized DNA molecules. Significant variability in the efficiency of modified nucleotide incorporation was observed, and attributed to the intrinsic properties of the modified nucleotides. Non-covalent attachment of streptavidin-coated nQDs to biotin-modified DNA synthesized using the primer extension method was observed by epifluorescence microscopy. Data regarding covalent attachment of nQDs to amine- and thiol-functionalized DNA was generally inconclusive; alternative characterization tools are necessary to fully evaluate these attachment methods. Full realization of this technology may facilitate new approaches to manufacturing materials at the nanoscale. In addition, composite nQD-DNA materials may serve as novel recognition elements in sensor devices, or be used as

diagnostic tools for forensic analyses. This report summarizes the results obtained over the course of this 1-year project.

Acknowledgments

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1.0 Introduction

The assembly of materials in living systems is driven by processes that are fundamentally different from those used to assemble synthetic materials. The active consumption of chemical energy enables living systems to create non-equilibrium structures and materials through stochastic assembly processes. These processes further allow for dynamic disassembly and reorganization of materials based on ever changing environmental conditions and physiological states. In addition to fundamentally unique assembly processes, living systems also possess a range of exquisite strategies for large-scale materials synthesis from molecular components. These strategies rely on the conversion of chemical energy into useful work, which in turn enable the mass production of functional, three-dimensional materials (e.g., proteins, DNA, cell membranes) with molecular precision [1]. Understanding and exploiting such energy-driven synthesis and assembly processes found in Nature may enable the development of new model systems for large-scale manufacturing of composite and/or synthetic nanomaterials.

In most living organisms, deoxyribose nucleic acid (DNA) encodes all of the information necessary for physiological existence (i.e., life). DNA molecules are synthesized/replicated through an energy-driven assembly process involving the stepwise, catalytic addition of DNA nucleotides by the enzyme DNA polymerase [1]. In cells, this process is used to generate duplicate copies of chromosomes during cell division. This replication process has been adapted for *in vitro* DNA replication, a method known as the polymerase chain reaction (PCR) [2]. Viewed from an manufacturing perspective, PCR represents a highly-efficient mass manufacturing protocol capable of generating 10^7-10^{10} DNA molecules within 2-4 hours, and with high fidelity (e.g., error rates of $<10^{-6}$) [3]. Because this technique enabled the *in vitro* amplification of virtually any DNA sequence, it quickly became a key driver in the biotechnology revolution in the 1990s.

Previous work at Sandia and elsewhere has demonstrated the ability to assemble metal and/or semiconductor nanocrystal quantum dots (nQDs) on DNA molecules [4-13]. The broad interest in utilizing DNA as a scaffold for organizing nanomaterials is based on a number of unique, intrinsic properties of DNA molecules. For example, the DNA contained in a single human chromosome, stretched end-to-end, can measure ~8 cm in length (chromosome 1; 2.46×10^8 base pairs) and 2 nm wide, an aspect ratio of 4×10^7. The polymeric nature of DNA also enables site-specific targeting of DNA base-pairs for attachment of nanomaterials. Each DNA strand is assembled from four sugar-phosphate nucleotide subunits: adenine (dATP), cytidine (dCTP), guanosine (dGTP), and thymidine (dTTP). Chemical modification of these nucleotides has previously been used to specifically link nanoparticles to the end of the DNA molecules [4, 11, 13]. Genetic and synthetic engineering of DNA sequences further enables pre-determined spatial organization and patterning of nanomaterials with atomic level resolution. Finally, the ability to mass manufacture DNA strands via PCR represents a key advantage to using DNA as a nanoscale scaffold; this aspect, however, has been relatively unexplored to date.

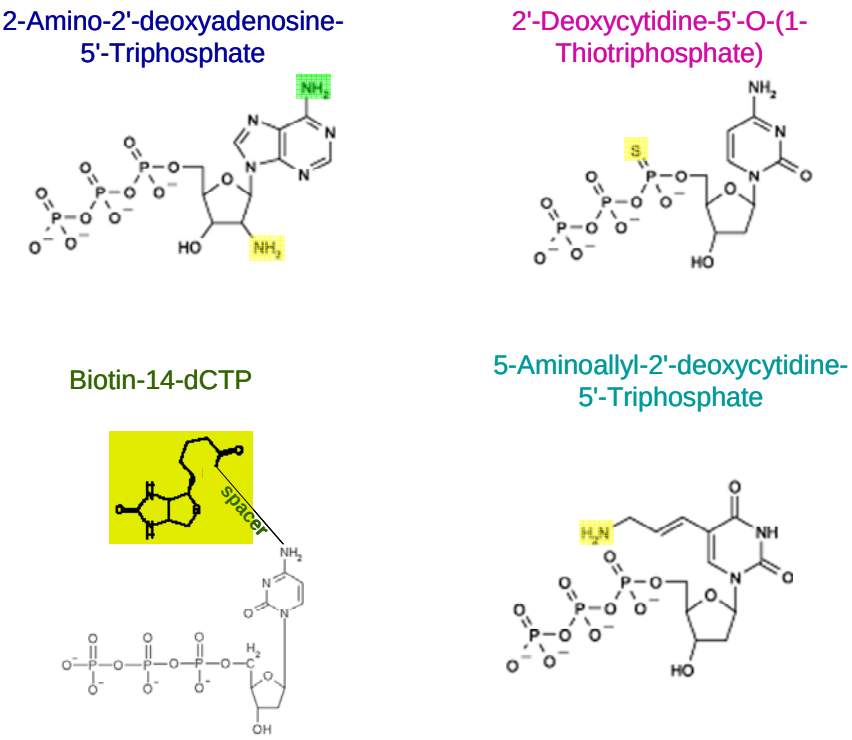

Figure 1: Molecular structure of modified DNA nucleotides used to link nanocrystal quantum dots to DNA strands. Functional groups highlighted in yellow represent primary targets for attachment; groups highlighted in green have secondary reactivity.

The long-term goal of the work presented in this report is focused on using DNA molecules and energy-driven replication for the assembly of nanocomposite materials. The specific objectives of this project were to: (1) develop methods for replicating DNA strands that incorporate nucleotides with "chemical handles," and (2) demonstrate attachment of nanocrystal quantum dots (nQDs) to functionalized DNA strands. It was hypothesized that nanocomposite materials of spatially-positioned nQDs can be synthesized using DNA scaffolds and *in vitro* replication methodologies. Achievement of these objectives will facilitate new approaches to manufacturing materials at the nanoscale. In addition, composite nQD-DNA materials may serve as novel recognition elements in sensor devices, or be used as diagnostic tools for forensic analyses. This report summarizes the results obtained over the course of this 1-year project.

2.0 General Strategy

The overall strategy involved a two-step process: (1) DNA is synthesized via PCR using modified DNA nucleotides, and (2) nQDs are attached to DNA molecules through linkage to modified nucleotides. To achieve attachment of nanoparticles to DNA, four

modified DNA nucleotides were chosen: 5-aminoallyl-2'-deoxycytidine-5'-triphosphate (aminoallyl-dCTP), 2-amino-2'-deoxyadenosine-5'-triphosphate (2-amino-dATP), 2'-deoxycytidine-5'-O-(1-thiotriphosphate) (thiol-dCTP), and biotin-14-dCTP (biotin-dCTP). The chemical structures and location of the functional groups are shown Figure 1. The nucleotides were chosen to evaluate a range of potential factors including chemical functionality (i.e., thiol, amine, and biotin), location of functional groups (i.e., phosphate backbone, sugar, nucleotide base), and nucleotide type (i.e., purine versus pyrimidine). In terms of attachment chemistry, nQDs may be linked to modified DNA nucleotides covalently through homo- or heterobifunctional crosslinkers (e.g., thiol-amine), or non-covalently through biotin-streptavidin linkage.

3.0 Incorporation of modified DNA nucleotides

Synthesis of chemically functionalized DNA molecules was achieved using the PCR methodology. DNA polymerase enzymes used in this process are able to discriminate between DNA and RNA nucleotides, a difference of a single hydroxyl group on the second carbon in the sugar backbone. This high intrinsic specificity, however, may also limit the efficiency of incorporating chemically modified nucleotides [14-17]. Previous studies have shown the ability to incorporate pyrimidine nucleotides with modifications at the 5-position of the nucleobase [14]. In addition, incorporation of modified nucleotides is a function of an interdependent relationship between the type of DNA polymerase and location of the modification [14, 16, 17]. In our experiments, it was hypothesized that varying the relative ratio of modified to unmodified nucleotides would alter the kinetics of the synthesis reaction, and permit incorporation of the modified nucleotides.

Materials & Methods

A circular DNA plasmid pET-Kin797, containing the coding sequence for a monomeric KIF1-like kinesin from *Thermomyces lanuginosus* was used as the DNA template for this study (Figure 2). This plasmid DNA was developed as part of a related project, and constructed by cloning the *Thermomyces* kinesin gene (2393 bp) into the pETBlue-1 plasmid vector from EMD Biosciences (3476 bp). *E. coli* strain NovaBlue (EMD Biosciences) was transformed with the pET-Kin797 construct, and cultured to obtain enough template DNA for the experiments.

The modified nucleotides 2-amino-dATP, aminoallyl-dCTP, and thiol-dCTP were obtained from TriLink BioTechnologies (San Diego, CA), and biotin-dCTP was obtained from Invitrogen Corp. (Carlsbad, CA). PCR Buffer, dATP, dCTP, dGTP, dTTP, $MgCl_2$ and native *Taq* Polymerase were obtained from Invitrogen Corp. (Carlsbad, CA). Corp. Native *Taq* Polymerase was chosen based on its lower fidelity compared with other DNA polymerase enzymes. DNA primers, Primer-3F: 5'-GCTGAATCCGTCGACAAAGG-3' (forward), and Primer-8R: 5'-GCTTCTTCCGGATGGTTGAA-3 (reverse) were synthesized by Sigma-Genosys (St. Louis, MO), and the thermal melting point (T_m) of these oligomers was calculated to be 54.6 and 54.1°C, respectively.

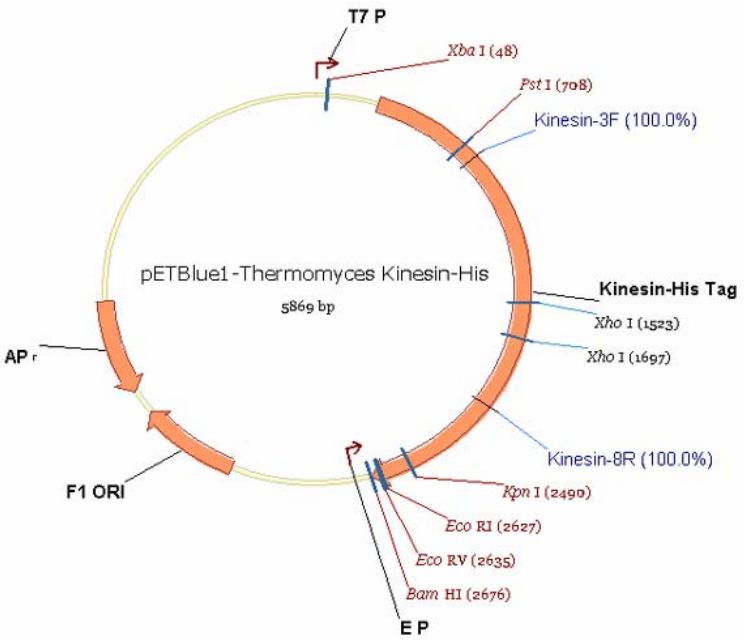

Figure 2: Plasmid map of the pETBlue-Kin797 construct used as a template for evaluating the incorporation of modified DNA nucleotides.

The goal of the initial experiments was to demonstrate and evaluate the incorporation of modified DNA nucleotides in the PCR reactions. Primers 3F and 8R were used to amplify a 1290-bp section (~450-nm long DNA molecule) of the *Thermomyces* kinesin gene. PCR reactions were prepared with final concentrations: 1X PCR Buffer, 0.2 mM dATP, 0.2 mM dCTP, 0.2 mM dGTP, 0.2 mM dTTP, 1.5 mM $MgCl_2$, 1 ng/µL pETBlue-Kin797 template DNA, and 1.0 U native *Taq* DNA Polymerase. To evaluate the incorporation of modified DNA nucleotides, the reactions were prepared with varying ratios of the modified dATP or dCTPs: 0% (control), 30%, 50%, 75%, 90%, and 100%. As an additional control, samples were prepared in which deionized water replaced the modified nucleotide. These control samples provided a baseline by which the relative efficiency of modified nucleotide incorporation could be calculated. The temperature cycling profile for the PCR reactions was: (1) 95°C for 2 min, (2) 95°C for 30 sec, (3) 55°C for 30 sec, (4) 72°C for 2 min, (5) 72°C for 20 min and (6) 4°C hold; steps 2-4 were cycled a total of 40 times. DNA was precipitated using 3M sodium acetate and 100% ethanol to purify the nucleic acid from the reaction components. Precipitated samples were analyzed on a 0.7% agarose gel, stained with ethidium bromide, and imaged under ultraviolet illumination with a GelLogic 100 camera (Kodak Corp). Kodak 1D software was used to calculate molecular weight and mass of each PCR band.

Results & Discussion

PCR amplified bands (molecular weight ~1300 bp) were observed for all modified DNA nucleotides, as shown in representative gel images (Figure 3). Reduction in the

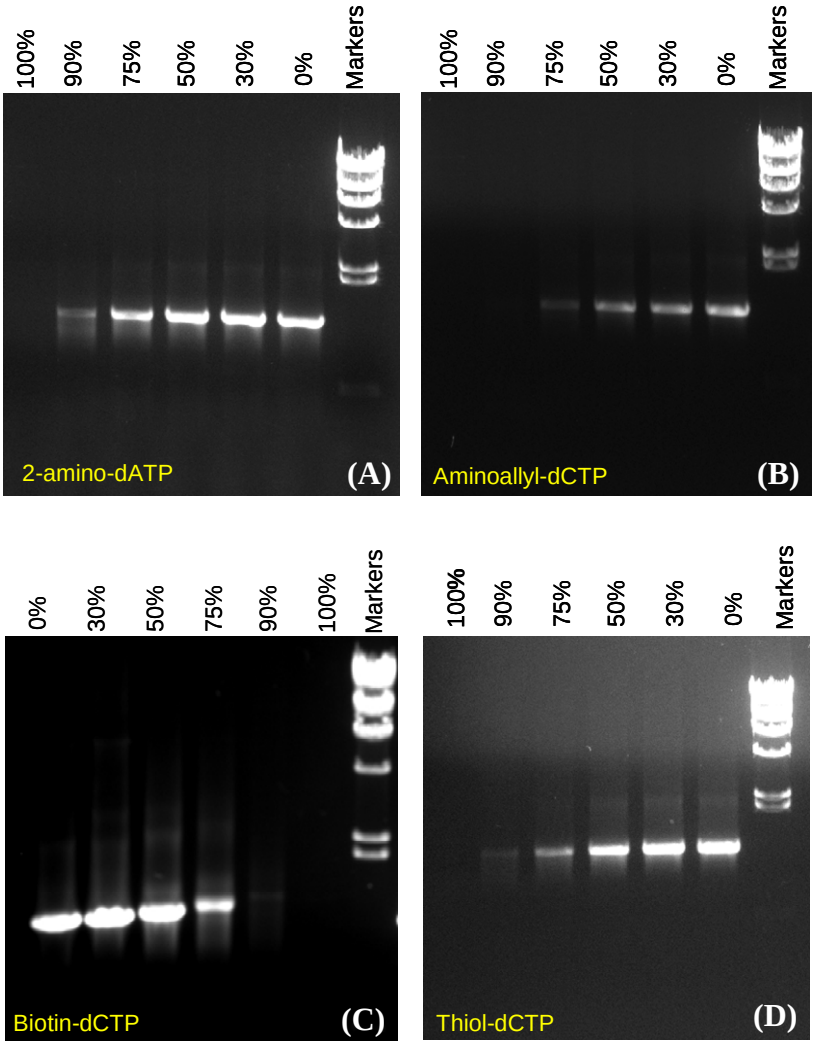

Figure 3: Agarose gel electrophoresis of DNA products amplified with the four different modified nucleotides: (**A**) 2-amino-dATP, (**B**) thiol-dCTP (**C**) biotin-dCTP, and (**D**) aminoallyl-dCTP. The percentages represent the percent of modified nucleotides; the marker lane is a Lambda-Hind*III* ladder (Invitrogen Corp).

mass of amplified DNA bands as a function of increasing percentages of modified nucleotide was clearly observable. Amplified bands were observed through the 90% level with all nucleotides, except the biotin-dCTP. Although all samples containing 100% modified dCTP, dATP, or water did not display visible bands, a low rate of incorporation may have occurred, but at a level below the detection sensitivity of gel electrophoresis. Similar reductions in the mass of amplified DNA bands was also observed for control samples in which deionized water was substituted for modified nucleotides.

The mass of all DNA bands were quantified based on the known mass of molecular weight standards, and used to determine the relative incorporation of the modified DNA

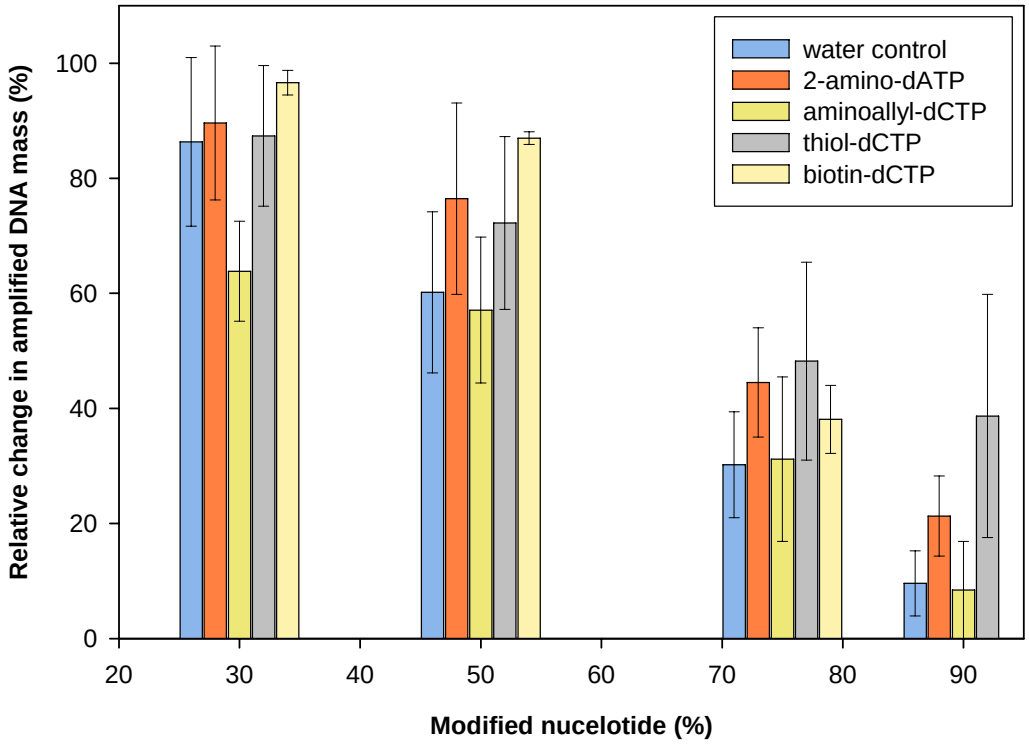

Figure 4: Average change in amplified DNA mass as a function of modified DNA nucleotides and water controls. The relative change was calculated using Equation 1 (below). Error bars represent the standard error of the mean.

nucleotides. Average masses were calculated from the triplicate samples for each PCR reaction. Although variability in the absolute band mass was observed among replicate samples, the general trend of a reduction in mass as a function of increasing percentages of modified nucleotide was consistent among replicate samples. The percent change in the mass of the amplified DNA product was calculated by dividing the mass of the amplified product for each sample containing modified nucleotides (i.e., 30, 50, 75, 90, and 100%) by the mass of the amplified product for samples with only unmodified nucleotides (i.e., 0%), as shown in Equation 1:

Equation 1:
$$\Delta x_{mass}(\%) = \frac{x_{\text{modified}}\,(ng)}{x_{\text{unmodified}}\,(ng)}$$

If the modified nucleotide was incorporated at the same rate, no change in the mass would be expected (i.e., $\Delta x_{\text{mass}} = 100\%$). Conversely, if the modified nucleotides were not incorporated at all, the reduction in the mass of the amplified product would be directly related to ratio of modified to unmodified nucleotide. Thus, water controls (i.e., substituting water for modified nucleotides at each ratio) were used to determine the expected change in mass in the event that the modified nucleotide was not incorporated.

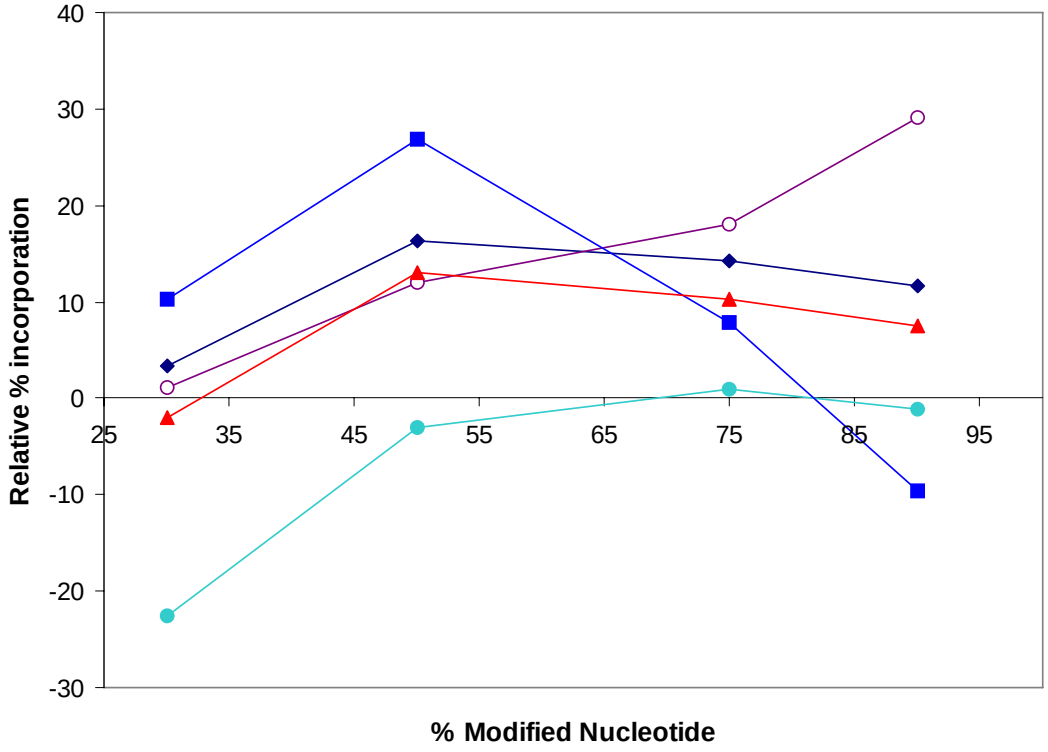

Figure 5: Relative percent incorporation of modified DNA nucleotides a function of the percent modified nucleotide present in the reaction. Aminoallyl-dCTP (–●–), biotin-dCTP (–■–), 2-amino-dATP (–♦–), thiol-dCTP (–○–), and the overall average (–▲–). The percent change in mass of the amplified DNA product was calculated based on control samples in which water was substituted for the modified nucleotide. See Equation 2 (below).

The average change in mass of amplified DNA was inversely proportional to the percentage of modified DNA nucleotide (Figure 4). Overall, the change in mass of the amplified DNA decreased at a relatively linear rate for the water control and modified nucleotides, except the biotin-dCTP. From these data, the relative percent incorporation could be calculated by subtracting the percent change in the mass associated with the water control from the percent change in the mass of each ratio of modified nucleotide, as shown in Equation 2:

Equation 2: $$incorporation(\%) = x_{\text{modified}}\,(\%) - x_{\text{water}}\,(\%)$$

Positive rates of incorporation were observed for 2-amino-dATP, thiol-dCTP, and biotin-dCTP across all percentages of modified nucleotide (Figure 5), suggesting that these modified nucleotides were successfully incorporated into newly synthesized strands. Conversely, the rate of aminoallyl-dCTP incorporation was consistently negative,

suggesting that this nucleotide inhibits the PCR reaction, and is not incorporated into newly synthesized strands (Figure 5). This inhibition/low incorporation rate is likely due to the location of the modification on the nucleotide. The aminoallyl group is attached to the cytidine base that is critical to the Watson-Crick base pairing in the DNA double helix. The presence of aminoallyl group on the nucleotide base may sterically interfere with the DNA structure and proper hydrogen bonding between complementary bases. The biotin group on the biotin-dCTP is also located on the cytidine base, but did not display the same inhibition observed for the aminoallyl-dCTP. Previous work has shown the incorporation of C5-position, modified pyrimidines is due to the location in the major groove of double-stranded DNA [16]. In addition to differences in the terminal functional group, the aminoallyl-dCTP and biotin-dCTP differ in the length of the spacer, 3 and 14 carbons, respectively (Figure 1). This length differential may help mitigate possible steric interference due to the functional group attached to the nucleotide base. The rigidity of the modified nucleobase chains have previously been shown to cause an increase in the incorporation efficiency in PCR reactions [15]. More specifically, modified nucleotides in which functional groups were linked to nucleobases through an alkyne chain displayed successful incorporation, whereas those linked through an alkane chain did not amplify in PCR [15]. The aminoallyl-dCTP is linked to the nucleobase through an alkene group, which should provide an intermediate level of rigidity compared with alkane and alkyne groups. The 2-amino-dATP and thiol-dCTP nucleotides were incorporated at rates between 10 and 25%, suggesting the functional groups on the sugar and phosphate units have minimal effect on DNA synthesis. Further, these data suggest that modified purine (i.e., dATP) and pyrimidine (i.e., dCTP) nucleotides are capable of being incorporated by the DNA polymerase enzyme.

Biotin-dCTP, aminoallyl-dCTP, and 2-amino-dATP displayed an initial increase in the relative rate of incorporation between the 30 and 50% levels, followed by a decrease in the rate between the 50 and 100% levels of modified nucleotide (Figure 5). The thiol-dCTP, however, displayed a monotonic increase in the rate of incorporation. This dependency of the incorporation rate on the percentage of modified nucleotide suggests that the ratio of modified to unmodified nucleotide can alter the ability of DNA polymerase to incorporate non-native nucleotides. The rate of incorporation peaked at the 50% level for the 2-amino-dATP and biotin-dCTP, 75% level for aminoallyl-dCTP, and 90% level for the thiol-dCTP (Figure 5). These data confirm a dependency on the ratio of modified to unmodified nucleotide, but also suggest that the relationship between the ratio of modified to unmodified nucleotide and the peak rate of incorporation is nucleotide specific.

4.0 Attachment of nanocrystal quantum dots

The second objective of this work was to demonstrate successful linkage of nQDs (or fluorophores) to modified DNA strands. From the results detailed above, amplified DNA molecules with biotin, amine, and thiol functional groups were generated by PCR. Based on these functional groups, nQD linkage to DNA can be achieved through covalent and non-covalent bonds (Figure 6). Linkage of nQDs to biotinylated DNA may be achieved

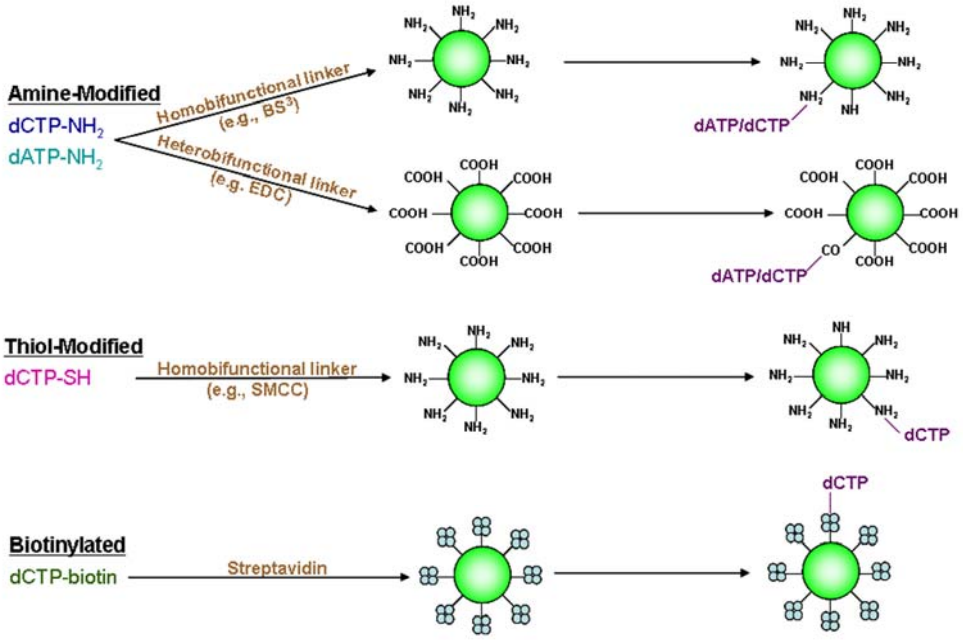

Figure 6: Scheme for attaching nQDs to modified DNA strands using covalent and non-covalent linkages.

using streptavidin to form a non-covalent bond with an extremely low disassociation constant, k_d = 4 x 10^{-14} M [18]. For both the amine and thiol modified DNA, covalent linkages can be achieved using homo- or heterobifunctional crosslinkers (Figure 6). Because the nQDs have multiple functional groups (~100 NH_2 (or COOH) groups/nQD; or ~5-10 streptavidin/nQD), a primary challenge to all linkages is ensuring that the multivalent nature does not cause aggregation of nQDs and DNA.

Materials & Methods

Amine functionalized Qdot®605 nQDs (Quantum Dot Corp., Hayward, CA) were attached to amine-modified DNA using the homobifunctional crosslinker bis(sulfosuccinimidyl)suberate (BS^3; Pierce Biotechnology Inc., Rockford, IL), a water-soluble analog of disuccinimidyl suberate (DSS). Amine functionalized nQDs in 0.1 M MES buffer (pH 7.0) were activated with an 87,000-fold molar excess of BS^3 for 30 min at room temperature. To remove excess crosslinker, activated nQDs were dialyzed against three exchanges of MES buffer. Activated nQDs were then reacted with 1.8 nmol or 1.9 nmol glycine, a primary amine donor, for 20 min at room temperature to deactivate excess NHS ester groups on the nQDs to percentages of 90% and 95%, respectively. Under these conditions, 5-10 activated NHS groups per nQD should be available for attachment of DNA. Modified DNA with the 2-amino-dATP was then reacted with the glycinated nQDs at a 250:1 stoichometric ratio.

The second approach involved linking amine-modified DNA to nQDs using the heterobifunctional crosslinker, succinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate (SMCC; Quantum Dot Corp., Hayward, CA), which links primary amine and thiol groups. To achieve this linkage, the amine functional groups on the nQDs were converted to thiol groups by reacting the nQDs with a 10,000-molar excess of N-succinimidyl-S-acetylthiopropionate (SATP; Pierce Biotechnology, Inc., Rockford, IL) for 30 min at room temperature in 50 mM sodium phosphate buffer with 1mM EDTA (pH 7.5). Excess SATP was removed by centrifuging thiol-modified nQDs in a Beckman AirFuge at 30 psi for 30 min; nQDs were resuspended in 250 μL of phosphate buffer and EDTA. The acetylated sulfhydryl groups were deprotected by adding 100 μL of 0.5 M hydroxylamine hydrochloride in 50 mM sodium phosphate, 25 mM EDTA (pH 7.5), and incubating the reaction at room temperature for 2 hr. Modified DNA with the 2-amino-dATP or aminoallyl-dCTP was reacted with an 80-molar excess of SMCC at room temperature for 1 hr with occasional mixing. Excess SMCC was removed through dialysis against three exchanges of 50 mM sodium phosphate (pH 7.4). The maleimide-activated DNA was then reacted with thiol functionalized nQDs overnight at 4ºC.

The third approach utilized non-covalent linkage of biotin-modified DNA to streptavidin-coated Qdot605® nQDs (Quantum Dot Corp., Hayward, CA). The biotinylated DNA (50 and 75% biotin-dCTP samples) was reacted with 0.05 picomoles of streptavidin nQDs for 30 min at room temperature with mild agitation. Samples in which deionized water was substituted for biotin-dCTP were used as a negative control.

In the last coupling approach, thiol-modified DNA was reacted with a maleimide-activated fluorescent dye. In the case of the thiol-dCTP, the thiol group on the phosphate chains exists as an equilibrium state of the tautomeric forms: thiol (-SH) and thione (S=O). The amplified DNA product was suspended in phosphate buffered saline (PBS; 8 mM Na_2HPO_4, and 2 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.4) with 1 μM dithiothreitol (DTT) for 30 min at room temperature to maximize the thiol state, and ensure efficient maleimide coupling. The thiol-modified DNA was then purified through a P-6 spin column (Bio-Rad Laboratories Inc., Hercules, CA), and subsequently reacted with a 20-molar excess of Alexa Fluor® 546 C_5 maleimide (Invitrogen Corp., Carlsbad, CA) at room temperature for 2 hr. The reaction was quenched with β-mercaptoethanol for 30 min at room temperature, and purified through dialysis against three exchanges of PBS.

All nQD-DNA and fluorophore-DNA reactions were analyzed by gel electrophoresis and epifluorescence microscopy. For gel electrophoresis, samples were separated on 0.7% agarose gel in 1x TBE, and visualized using UV excitation. Epifluoresence microscopy was performed at 100x magnification with an Olympus IX-71 inverted microscope equipped with a Hamamatsu Orca CCD camera.

In the final experiment, 15-micron long nQD-DNA composites were formed using Lambda DNA as a template. The method for synthesizing these 48,502-bp DNA templates relied on the primer extension method in which thirteen oligomer primers, complementary to the plus-sense DNA strand (i.e., coding strand), were used as seeds to "extend" the negative-sense DNA strand and brigde the individual primers (Figure 7). The sequences of the oligomers were: λ1 (5'-CGTTCTTCTTCGTCATAACTTAATG-

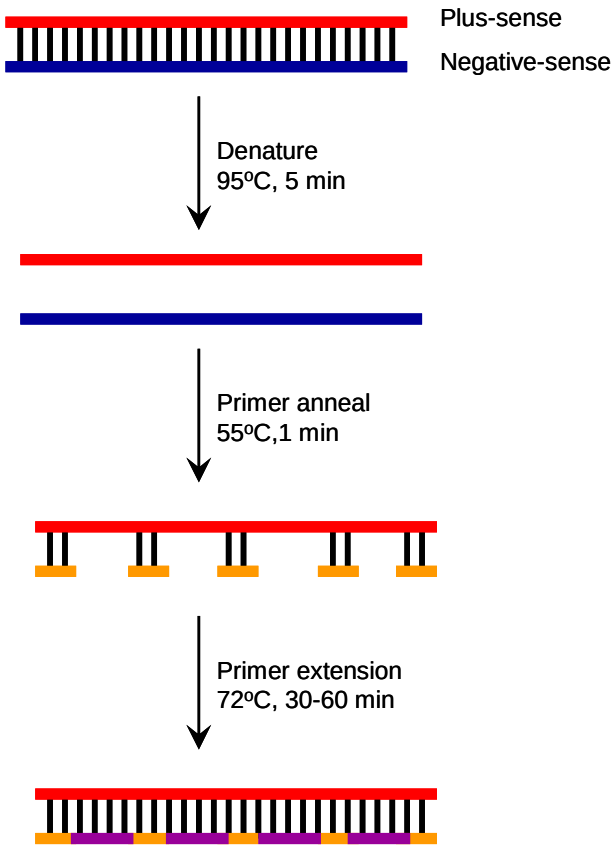

Figure 7: Schematic representation of the primer extension method used to synthesize 48,000-bp Lambda DNA templates. Double-stranded DNA (red and blue) is initially thermal denatured at 95ºC; oligomer primers (gold) are subsequently annealed to one of the DNA strands. *Taq* polymerase then synthesizes a new DNA strand from these oligomer primers.

3'), λ2 (5'-CTGGAAGAGGCCATCGTTCG-3'), λ#3 (5'-TTGCTTCATCCGCGATATCG-3'), λ4 (5'-AGTCAGTTCGCGGTACTGGAGGAGG-3'), λ5 (5'-CAGAACAAAACGCTCTGGTC-3'), λ6 (5'-ACTGATGCAACTGACTCAGC-3'), λ7 (5'-GGCTTGGCTCTGCTAACACGTTG-3'), λ8, 5'-GCCAAGAATCTCTTTGCATTTA-3'), λ9 (5'-GGCCTGTGTCAGTTCTGACG-3'), λ10 (5'-CAAGGCAGCAATCAGGATTG-3'), λ11 (5'-CTTACTGATGCGGAATTACG-3'), λ12 (5'-ATCTGATGCAATTTGCACAC-3'), and λ13 (5'-GGAAGAACGCGGGATGTTCA-3'). A 10 μM solution containing each of these primers was prepared in deionized water. Streptavidin-nQDs in 1x PCR buffer was reacted with 640,000-fold molar excess of biotin-dCTP at room temperature for 20 min. This reaction mixture was then added to a primer extension reaction containing 1x PCR SuperMix (Invitrogen Corp., Carlsbad, CA), the primer mixture (20 μM final concentration), and 10 μg/mL of Lambda DNA. Deionized water was

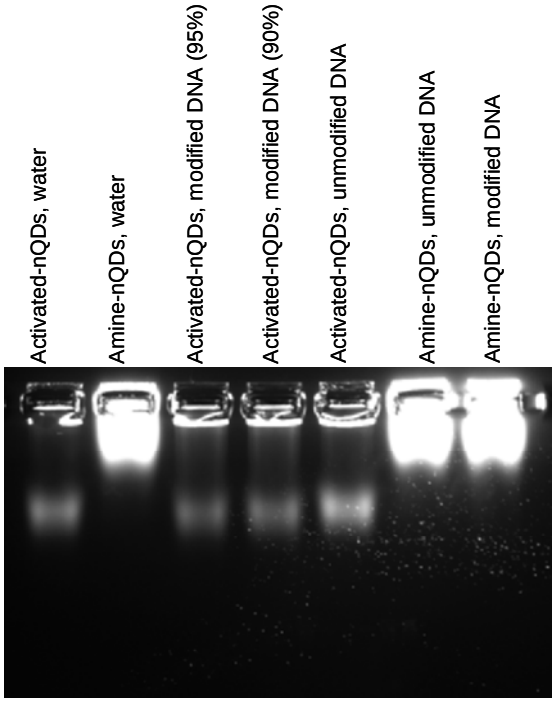

Figure 8: Agarose gel electrophoresis of amine-functionalized and NHS-ester activated nQDs following reaction with modified and unmodified DNA, or water.

substituted for the nQD-dCTP reaction as a control sample. The synthesis reaction was carried out as shown in Figure 7. Control and nQD-DNA samples were visualized by epifluorescence microscopy following synthesis.

Results & Discussion

Overall the results of linking nQDs and fluorophores to modified PCR DNA products were inconclusive. Figure 8 shows representative results of these experiments. A clear difference in the electrophoretic mobility of amine-functionalized and NHS-ester activated nQDs was observed (Figure 8). The increased mobility of the activated nQDs suggests a change in the net charge of the nQDs based on activation with the BS^3 crosslinker. No difference, however, in the mobility between NHS-ester activated nQDs reacted with modified DNA, unmodified DNA, or water was observed (Figure 8). This may be explained if either (1) no DNA is attached to the nQDs, or (2) the attached DNA does not substantially alter the electrophoretic mobility of the nQDs. Thus, no direct conclusions can be reached with respect to linking nQDs to DNA through the amine-modified dATPs or dCTPs. Similar results were obtained for biotin-streptavidin linkage of nQDs to DNA, and fluorophore attachment to thiol-modified DNA (*data not shown*). Epifluorescence microscopy of these samples was also inconclusive. Small clusters of nQDs were observed in DNA samples with modified nucleotides, but in very low

Figure 9: Fluorescence photomicrograph of amine-modified DNA reacted with nQDs through crosslinking with BS^3.

frequency (Figure 9). The stretched length of the PCR DNA products is ~450 nm, which is close to the resolution of the optical microscopy. Therefore, little conclusions can be reached as to the nature of these structures. High resolution imaging (e.g., TEM) may enable further characterization of these structures, and potentially an understanding of whether the nQDs were attached to DNA molecules.

Attachment of streptavidin-coated nQDs to DNA was successful using the primer extension protocol with biotin-dCTP. Linear strands of DNA with attached nQDs were observed by epifluorescence microscopy (Figure 10). Because the Lambda DNA template is significantly longer than the PCR amplified DNA (48,502 bp versus 1280 bp), even low biotin-dCTP incorporation rates may enable attachment of enough nQDs for visualization. In general, an average of 3-8 nQDs were observed per DNA strand, whereas the Lambda DNA contains >12,000 dCTP residues. Thus, the overall rate of modified nucleotide incorporation and nQD attachment is ~0.04%. The PCR results (Figure 5) suggest that biotin-dCTP is incorporated at a rate of 5 – 25% at moderate ratios of modified and unmodified nucleotides. In these experiments, the ratio of modified to unmodified nucleotides was low (i.e., <5%); thus, a low incorporation rate is expected based on the selectivity of the *Taq* polymerase [17, 19-21]. These results also suggest that the primer extension methodology may be adapted for incorporating modified nucleotides, and subsequent attachment of nanoparticles. Work by Jager *et al.* [22] demonstrated the incorporation of eight modified DNA nucleotides using primer extension, and may serve as a foundation for further exploring this methodology for assembling nanoparticle arrays.

5.0 Conclusions

Successful incorporation of amine-, thiol-, and biotin-functional groups was demonstrated using PCR-based DNA amplifications processes. Significant differences in the efficiency of incorporating the different modified groups was observed, and likely

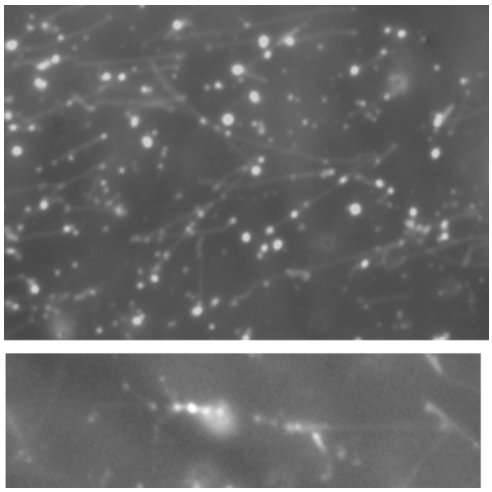

Figure 10: Photomicrographs of gel electrophoresis of nQD-DNA assemblies formed using the primer extension method and Lambda DNA templates. DNA was stained with ethidium bromide to permit co-visualization of both nQDs and DNA.

attributable to the position and rigidity of the linkers. Previous work with PCR amplification using modified nucleotides suggests that positioning the linker at the 5-position of the nucleobases enables efficient incorporation [14]. Successful incorporation of nucleotides modified at other positions has also been demonstrated [14-17]. The efficiency of incorporation, however, displayed a strong relationship to the type of DNA polymerase enzyme used for synthesis [14, 16, 17]. In general, DNA polymerases that lack exonuclease activity are preferable based on lack of proof-reading ability, and increased likelihood of modified nucleotide substitution [17]. In the current study, the native *Taq* DNA polymerase used for PCR and primer extension reaction lacks 3'→5'exonculease activity, and should incorporate the modified nucleotide with moderate efficiency. Further investigation of the wide variety of commercially available DNA polymerases, however, is necessary to fully maximize the "manufacturing process" of chemically-functional DNA scaffolds.

Despite evidence supporting the successful incorporation of modified nucleotides in DNA scaffolds, no or limited attachment of nQDs and molecular dyes to modified DNA was observed. Electrophoretic and microscopic analyses of nQD-DNA reactions using PCR-amplified DNA were inconclusive. Composite nQDs/biotin-modified Lambda DNA structures, however, were observed by epifluoresence microscopy, providing the strongest evidence of successful non-covalent linkage between nQDs and DNA. Attachment of nanoparticles to DNA oligomers and double-strand DNA molecules has previously been achieved using non-covalent, biotin-streptavidin linkages [23]. Covalent linkage of metal and semiconductor nanoparticles has been demonstrated [8, 9, 23-25], but often limited to derivatized DNA "ends" and oligomers. Further optimization of the conjugation chemistry is necessary to achieve a robust method for linking DNA to

internally located nucleotides. Further, a fundamental challenge that must be addressed with respect to both covalent and non-covalent approaches is achieving a single linkage between DNA nucleotides and nQDs.

In summary, the general goal of this work was to exploit DNA replication technology to manufacture chemically-active DNA scaffolds for assembling semiconductor nanoparticle arrays. The long-term application of this work centers on the energy-driven assembly of photoactive materials with a range of materials science-, nanoelectronics-, and nanophotonics-based applications. There has been considerable interest in using biological molecules as scaffolds for synthesizing and assembling nanoscale materials [26-34]. To date, the majority of work has focused on using biomolecules as a static scaffold for assembling composite materials. The exploitation of energy-driven assembly processes may enable novel manufacturing strategies that incorporate bio-mimetic or inspired processes. In addition to nanomaterial-based applications, nQD-DNA composites may be widely applied in biomolecular applications such as live cell imaging as an alternative to fluorescent dyes [4, 8, 11, 35-38]. nQDs offer several unique properties including high quantum yield, increased photo-stability, and broad spectrum excitation, which has lead to their application in many biomolecular assays [12, 39]. Overall, the ability to synthesize DNA scaffolds with multiple functional “handles” was realized. Attachment of nQDs to these scaffolds, however, was observed for Lambda DNA scaffolds, but was inconclusive for PCR-amplified DNA scaffolds; further investigation is required to fully understand and optimize the attachment chemistries necessary to achieve high density attachment of nQDs to DNA.

6.0 References

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