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ARTICLE

Synthetic Approaches for Copolymers Containing Nucleic Acids and Analogues: Challenges and Opportunities

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A deep integration of nucleic acids with other classes of materials have become the basis of many useful technologies. Among these biohybrids, nucleic acid-containing copolymers has seen rapid development in both chemistry and application. This review focuses on the various synthetic approaches to access nucleic acid-polymer biohybrids spanning post-polymerization conjugation, nucleic acids in polymerization, solid-phase synthesis, and nucleoside/nucleobase-functionalized polymers. We highlight the challenges associated with working with nucleic acids with each approach and the ingenuity of the solutions, with the hope of lowering the entry barrier and inpsiring further investigations in this exciting area.

Introduction

Nucleic acids are a brilliant gift from *Nature*. Since the elucidation of the B-form DNA structure in the 1953 landmark paper by Watson and Crick,[1] synthetic nucleic acids and their analogues have populated many fields of explorations including materials science,[2] nanotechnology,[3] and medicine.[4] The diversity of their application reflects the powerful traits exhibited by these materials: highly predictable and programmable base pairing, ability to work with a plethora of natural or engineered enzymes, and unique photochemical and energy transfer properties. Key to many of these applications is the ability to interface nucleic acids with other types of materials, either covalently or non-covalently. For example, oligonucleotides are used to decorate gold nanoparticles via the thiol-gold bond to form "spherical nucleic acids" (SNAs), which are being investigated as "atom equivalents" in crystal engineering and as therapeutic/prophylactic agents for disease treatment/prevention.[5, 6]

One fruitful area of nucleic acid-materials integration involves the formation of different classes of copolymers – block copolymers, stars, bottlebrushes, crosslinked networks, etc. Pegaptanib,[7, 8] a Y-shaped poly(ethylene glycol) (PEG)-oligonucleotide conjugate, was approved in 2004 by the U.S. Food and Drug Administration for the treatment of age-related macular degeneration, where the PEG component serves to improve ocular retention after intravitreal injection. Similar linear PEG conjugates are also being used as supports in liquid-phase oligonucleotide synthesis.[9] While these simple conjugates, characterized as having a hydrophilic polymer component and a single oligonucleotide strand, are relatively straightforward to synthesize, the synthetic methodology for more architecturally complex copolymers and amphiphilic copolymers, which are oftentimes at the forefront of scientific and technological explorations, is far from trivial. Workarounds are often needed to circumvent the difficulties associated with nucleic acids. For example, nucleic acid is inherently highly anionic due to the charged phosphate backbone and therefore generally lacks solubility in organic solvents,[10, 11] which puts a severe limitation on the chemistries that can be used to assemble the copolymers. Additionally, the nucleobases contain nucleophilic exocyclic amines and basic aromatic nitrogen atoms, which may disrupt transition metal catalysts. Nucleotides are also subject to damage due to various reactivities such as acid depurination, oxidation (e.g. by $Cu(I)/O_2$), reaction with strong nucleophiles/electrophiles, reaction with radicals, photodimerization, etc.[12, 13] Consequently, a variety of strategies have been developed to access useful copolymers containing nucleic acids. This review will summarize these strategies, focusing on the chemistries used, structural features of the resulting copolymer, and challenges faced/overcome.

Post-Polymerization Modification

The earliest development of nucleic acid-containing polymers primarily involves the "grafting-onto" strategy, i.e. direct conjugation the nucleic acid to the synthetic polymer (Table 1). This strategy may allow the polymer and the nucleic acid to be separately prepared, purified, and fully characterized prior to the coupling reaction. However, removal of unreacted nucleic acid and/or the polymer after the conjugation can be difficult, requiring various chromatography protocols (size exclusion, reversed-phase, anionic exchange, etc) to be developed. Additionally, a severe limitation involves the requirement for the polymer component to be soluble in water or a mixed

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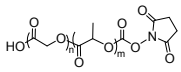
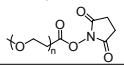
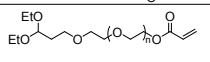
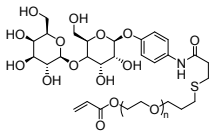
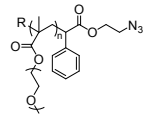
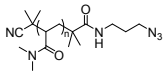
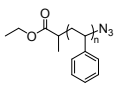
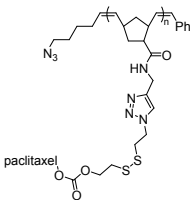
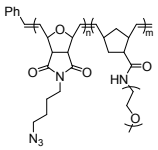
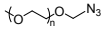
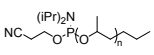
solvent with water content for homogeneous coupling, as unmodified DNA or RNA has limited solubility in almost all organic solvents.[10, 14, 15] Therefore, highly hydrophobic, non-polar polymers are generally thought to be incompatible for conjugation with DNA, although extensive optimizations have achieved reasonable yields.[16]

With regard to the bioconjugation reaction, amidation is among the first been studied. Park and co-workers coupled poly(D,L-lactic-*co*-glycolic acid) (PLGA) with an antisense oligonucleotide to form an amphiphilic diblock copolymer, which self-assembled into micelles in aqueous buffer.[17] The reaction was carried out between 5' primary amine-terminated oligonucleotide and the ω-carboxylic acid of PLGA under the dicyclohexylcarbodiimide/*N*-hydroxysuccinimide (DCC/NHS) condition in a predominantly dimethyl sulfoxide (DMSO) solution with 70% conjugation efficiency. The same group also synthesized poly(ethylene glycol) (PEG)-oligonucleotide conjugates using NHS-activated PEG and 5' amine-derivatized oligonucleotide in sodium phosphate buffer with 65% yield after HPLC.[18, 19] For nucleic acids lacking a 5' amine, one can be added by reacting the 5' phosphate with ethylene diamine in the presence of a carbodiimide and imidazole.[18]

Aside from amidation, thiol-maleimide and thiol-acrylate(mide) Michael addition reactions have also been used to synthesize

Table 1. Examples of nucleic acid-polymer conjugates synthesized via post-polymerization conjugation.

Reaction Type	Polymer Structure	Polymer M_n (kDa)	Nucleic Acid	Conversion (%)	Reaction Condition	Reference
Amidation		10	15-mer DNA terminal amine	75	Sodium borate buffer (pH 8.0) DMSO, R.T., 12h	[17]
		2	18-mer DNA terminal amine	65	Phosphate buffer (pH 7.0) R.T., 1.5 h	[18], [19]
Michael addition		4.8	19-mer DNA 3'-thiol	63	10 mM Tris-HCl buffer (pH 8.0)	[20]
		5.1	17-mer DNA/RNA 3'-thiol	79	10 mM Tris-HCl buffer (pH 8.0) R.T.	[21], [22]
CuAAC		167	18-21-mer DNA terminal alkyne	-	Tris buffer (pH 8.0) Sodium ascorbate acid, ACN	[28]
		122	63-mer DNA terminal alkyne	79-90	Iodo(triethyl phosphite)copper(I) DMF or DMSO or NMP, R.T., overnight	[31]
		3.9-14	6-26-mer DNA on CPG 5' alkyne	56-99	CuI/DIPEA/acetic acid DCM, R.T., 8 h	[44]
SPAAC		10.8	21-mer DNA 5' DBCO	40	Water/DMSO, 40 ºC, 48 h	[34]
		178.8 285.5	18-21-mer DNA/RNA 5' DBCO	>99	2 M NaCl/water, 40 ºC, 48 h	[35], [36] [38], [39]
		10.0	41-58-mer hairpin DNA terminal DBCO	-	3 M NaCl/water, 40 ºC, 48 h	[37]
Phosphoramidite		1-6.8	22-mer DNA on CPG	41-32	Standard DNA synthesis with extra (1 min) coupling time	[42], [43]

DNA-containing copolymers. Kataoka developed a series of oligonucleotide-PEG conjugates with varying substituents at the α-terminus of the PEG.[20-22] The Michael reaction between thiol-terminated oligonucleotide and acrylate-modified PEG results in an acid-labile linkage (β-propionate), while reaction with a maleimide gives a non-releasable thioether, both in 60-80% yields. Of note, the thioether linkages are subject to β-elimination (retro-Michael) reactions under basic conditions, and thus should be avoided if subsequent treatment with certain bases (NaOH, ammonia, etc) is needed.[23] Additionally, strong Michael acceptors (e.g. acrylonitrile) may also alkylate sites of the nucleobases (e.g. N3 of thymine); the reaction is often a source of impurity that takes place during the deprotection step of oligonucleotide chemical synthesis.[24, 25] Other than thiols, amine-nucleophiles have also been used to prepare nucleic acid-polymer conjugates via amination. For example, Nguyen and co-workers conjugated amine-terminated oligonucleotides to amphiphilic block copolymer micelles with surface-exposed tosylates, producing SNA-like structures.[26] Because the SNAs are the desired final product, yields for the conjugation reaction were not reported.

The discovery of copper(I)-catalyzed and strain-promoted azide-alkyne cycloaddition (CuAAC and SPAAC) click chemistry paved a new road to bioconjugates.[27, 28] The reaction boasts very high selectivity and reaction yields. Matyjaszewski and Das have used CuAAC to synthesize a multiarm star conjugate consisting of polyacrylate-*g*-oligo(ethylene oxide) arms and multiple DNA strands on the periphery of the star.[29] The conjugation was performed in aqueous buffer using an optimized "ligandless" condition, with near-quantitative yield as suggested by the loss of azide vibrations in infrared (IR) spectroscopy.[30] O'Reilly et al conducted a comprehensive optimization study for the CuAAC conjugation between hydrophobic polymers and a 22-mer oligonucleotide, and achieved exceptional yields (>50%) for permanently hydrophobic polymers such as polystyrene, poly(dimethylacrylamide), and poly(4-acryloylmorpholine).[31] It is suggested that the record-breaking yields are in part due to the use of copper iodide triethylphosphite as the catalyst ($CuI•P(OEt)_3$), which has a pre-complexed copper(I) that increases reaction efficiency relative to traditional catalysts which require *in situ* formation of the complex. Of note, prolonged treatment of DNA or RNA with Cu(I) can lead to extensive oxidative lesions mediated by reactive oxygen species such as hydroxyl radicals. However, the damage can be dramatically suppressed by introducing DMSO, a known radical scavenger, to the reaction system.[32] Alternatively, the oxidation issue can be completely bypassed by switching to SPAAC, where ring strain is used to promote cycloaddition instead of Cu(I). Our group have routinely used this reaction to conjugate a variety of dibenzocyclooctyne (DBCO)-modified nucleic acids and analogues to azide-derivatized polymers, even hydrophobic ones,[33, 34] often with near-quantitative yields.[35-39]

One challenge associated with bioconjugation is the removal of unreacted polymer and nucleic acid. Mirkin and co-workers circumvented this difficulty by performing the conjugation reaction on the solid support. The group converted hydroxy-terminated polystyrene to a phosphoramidite by reacting it with chlorophosphoramidite.[40] The resulting polymer was incorporated into the DNA during solid-phase synthesis (SPS), and unreacted polymers were simply removed during the washing step. When polymers bearing multiple phosphoramidite functionalities are used during SPS, polyvalent DNA-polymer conjugates are expected. Indeed, Mirkin and Nguyen found that such a strategy can achieve up to 30% occupation of the potential polymer attachment sites by DNA strands.[41] The Herrmann group also adopted the solid-state reaction in the preparation of a series of DNA-*b*-poly(propylene oxide) (PPO) diblock copolymers, which can form well-ordered structures in aqueous buffer due to amphiphilicity.[42,43] Along the same line, our group have performed solid-phase conjugation using the CuAAC click reaction to obtain a library of polystyrene-*b*-DNA and poly(tBu acrylate)-*b*-DNA conjugates with varying DNA and polymer lengths.[44] While quantitative yields were achieve with very short DNA (6-mer), longer DNA and polymer lengths decrease the yield, likely due to limited pore size of the controlled pore glass support (CPG), with the lowest yield (56%) observed for a 26-mer DNA and a 14 kDa polystyrene. One advantage of this strategy is that failed DNA strands during SPS will not be coupled to the polymer, as they lack the 5' functionality used for coupling (assuming 3'-5' DNA SPS). This feature saves the traditional purification step needed for the DNA.

Nucleic Acids as Initiator or Macromonomer for Polymerization

While bioconjugate techniques have improved the accessibility of nucleic acid-containing copolymers considerably, certain constructs, such as high-density (e.g. 100% grafting density) multivalent DNA conjugates and amphiphilic conjugates, are still difficult to achieve using a post-polymerization conjugation methodology due to the strong repulsive interaction of the negatively charged phosphates in the nucleic acid backbone. Thus, methods are being developed to directly involve nucleic acids in polymerization reaction and thereby access new types of nucleic acid-containing biomaterials.

The He group studied living polymerization using DNA as a macroinitiator (Figure 1A).[45, 46] The initiator DNA strand is immobilized on a surface by hybridization to a capture strand. The anchored dsDNA macroinitiator then initiates the polymerization of hydroxyethyl methacrylate (HEMA) in water in the presence of Cu(I). The growth of the surface brush can be visualized as a change in substrate opacity, which serves as a means for signal detection. Interestingly, polymer growth was accelerated with DNA, possibly due to the charged backbone promoting Cu(I) association. Matyjaszewski and Das expanded this methodology by developing an ATRP initiator phosphoramidite, allowing for the initiating site(s) to be incorporated anywhere in the oligonucleotide sequence (Figure

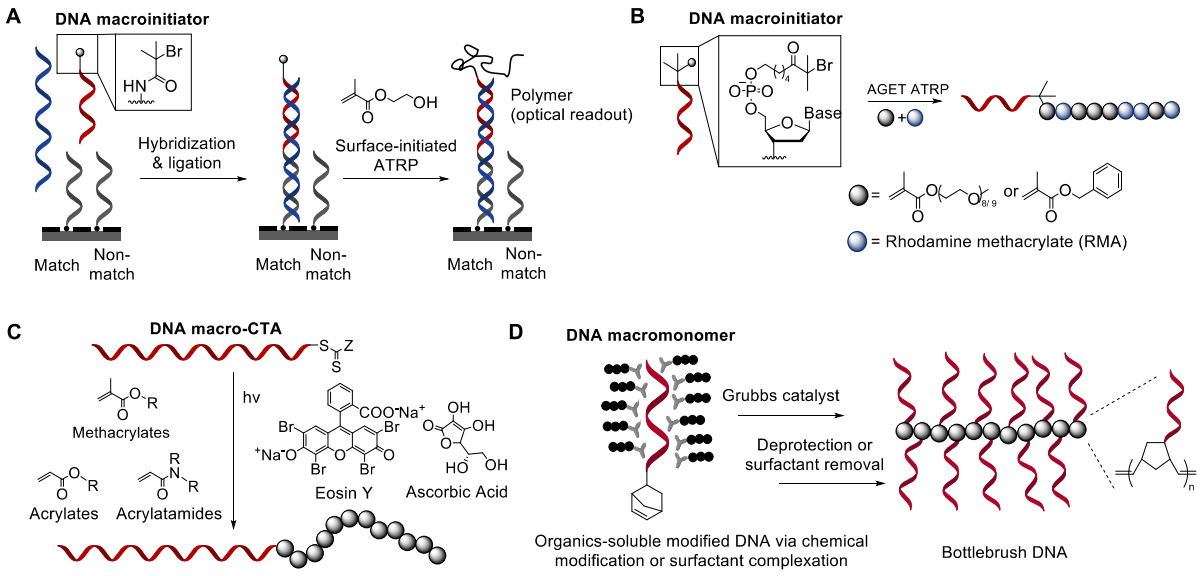


Figure 1. Living polymerization approaches to nucleic acids-polymer conjugates. The nucleic acid can be a macroinitiator for **A)** surface-initiated ATRP[42] or **B)** solution-phase ATRP, [45] a macro-CTA for **C)** RAFT polymerization,[50] and a macromonomer for **D)** ROMP.[23, 52, 53]

1B).[47, 48] The polymerization reaction can then be performed from the DNA macroinitiator, either in solution or off the solid support.

In order to minimize DNA damage from Cu(I), raise oxygen tolerance, and enable better control of the polymerization reaction, improved ATRP methods such as activator generated by electron transfer (AGET) ATRP and photochemically mediated ATRP (photoATRP) have been applied in the synthesis of DNA-containing copolymers. For example, He and co-workers enhanced their DNA detection system with AGET ATRP by using ascorbic acid as the reducing agent for Cu(II), allowing for a lower concentration of Cu(II) (0.3 mM) to be used.[49] Maynard *et al.* reported a series of siRNA-polymer conjugates that were synthesized by AGET ATRP of two monomers, poly(ethylene glycol) methyl ether methacrylate (PEGMA) and di(ethylene glycol) methyl ether methacrylate (DEGMA) via the grafting-from method.[50] However, only ~30-50% yield was observed, which is lower than the grafting-onto method (~50-80%, compared side-by-side). Matyjaszewski and co-workers developed an automated system for photoATRP by using a DNA synthesizer.[47] After the irradiation of UV light (365 nm), copper(II) was photo-reduced in the presence of electron-donor ligands to initiate the reaction. The reaction only requires ppm levels of a copper catalyst at ambient temperature, and can be carried out by non-experts in synthetic polymer chemistry to obtain DNA-polymer conjugates without degassing procedures.

The general grafting-from method has been expanded to include reversible addition-fragmentation chain transfer (RAFT) polymerization, where the oligonucleotide is attached a RAFT chain transfer agent (CTA). For instance, He and coworkers polymerized oligo(ethylene glycol) methacrylate (OEGMA) monomers via surface-anchored, trithiobarbonate-derivatized DNA, and demonstrated successful growth by ellipsometric and IR measurements.[51, 52] Similarly, Weil et al reported a solution-phase photoinduced RAFT polymerization to synthesize DNA-polymer hybrids (Figure 1C).[53] The DNA macro-CTAs were synthesized by coupling tri- and dithiol carbonate-based CTAs to amine-terminated, single-stranded DNA. Upon irradiation by blue light (470 nm) in the presence of the photocatalyst, Eosin Y, and ascorbic acid, three monomers families (i.e., methacrylates, acrylates, and acrylamides) were successfully polymerized onto the DNA.

Apart from using nucleic acid as an initiator in polymerization, a limited number of successes were achieved in directly polymerizing oligonucleotides as a macromonomer. Nucleic acid-based macromonomers present a significant difficulty for polymerization. Not only must the oligonucleotide be soluble in the solvent of choice, the polymerized product must also be soluble. Additionally, oligonucleotides are bulky and highly anionic, resulting in steric issues during propagation. Gianneschi and co-workers solved these problems by using a non-charged DNA analogue, peptide nucleic acid (PNA), as the macromonomer, which have great solubility in dimethylformamide (DMF). The team was able to polymerize a norbornene-functionalized decamer PNA by ring-opening metathesis polymerization (ROMP) using 3rd generation Grubbs catalyst, achieving quantitative yields in some instances.[54] Herrmann et al was the first to achieve ROMP of natural, phosphodiester-based DNA macromonomers. To overcome the problems associated with solubility and charge-repulsion, Herrmann used a cationic surfactant, didodecyldimethylammonium bromide (DDAB), to form an electrostatic complex with a 7- or 14-mer oligonucleotide in aqueous solution. The isolated complex is dried and then redissolved in an organic solvent such as DMF, DMSO, tetrahydrofuran (THF), or chloroform, where polymerization was carried out (Figure 1D).[55] The strategy works well not only

for polymerization but also for coupling reactions with hydrophobic ligands. However, complete removal of the surfactant from the product may be difficult for applications that require high purity. Our group also approach this problem, albeit from a different angle. Instead of using surfactants to neutralize the negative charge associated with DNA, we recognized that typical oligonucleotides are synthesized with various protecting group attached to the exocyclic amine of nucleobases and the phosphates in the triester form, which render the oligonucleotide highly hydrophobic and charge-neutral. The protected form of DNA (protDNA) can be removed from the solid CPG support without affecting the protecting groups using triphenylphosphine cleavage of a disulfide linker, and the isolated protDNA is soluble in dichloromethane. ROMP of a 15-mer protDNA modified with a terminal norbornene yielded a bottlebrush-type conjugate with 70-90% yields and high molecular weight (highest M_n: ~300 kDa, Figure 1D).[23] After the polymerization, treatment with methanolic ammonia for 4 h cleanly removes the protecting groups. Collectively, substantial progress has been made to integrate nucleic acids with polymerization. With the vast knowledge base of both polymer chemistry and nucleic acid chemistry, we anticipate that a deeper merge of the two fields will greatly expand current materials possibilities.

Solid-Phase Synthesis

Instead of relying on polymerization to generate the polymer component of the nucleic acid-polymer biohybrid, it is also possible to use solid-phase reactions, typically used for oligonucleotide synthesis, to assemble the polymer. By designing appropriate phosphoramidite monomers, non-nucleotide units can be easily incorporated. In contrast to polymerization, SPS is carried out in a stepwise, iterative fashion, where the addition of each monomer involves a set of deprotection, coupling, capping, and oxidation steps. However, the complex synthesis can be automated, and the result is unmatched control in monomer sequence, degree of polymerization, polydispersity, and relative position of DNA on the polymer chain. Nonetheless, while SPS may be ideal for linear or slightly branched DNA-polymer conjugates, multivalent architectures (i.e. multiple oligonucleotide strands) such as stars and brushes, are currently not accessible via SPS. In addition, if phosphoramidite chemistry is to be used for the SPS, the monomers must be free of incompatible functionalities such as unprotected amines and hydroxyl groups, and the resulting polymer segment will have a phosphorous-containing backbone. Finally, the size of the DNA-polymer conjugate is limited due to the exponentially decreasing yields as coupling number increases. For example, for a combined length of the nucleotides and polymer repeat units of 50, even when each coupling step enjoys 98% yield, the overall yield is a mere 36%.

Early work in this field revolves around introducing functional residues to the oligonucleotides to improve their properties.

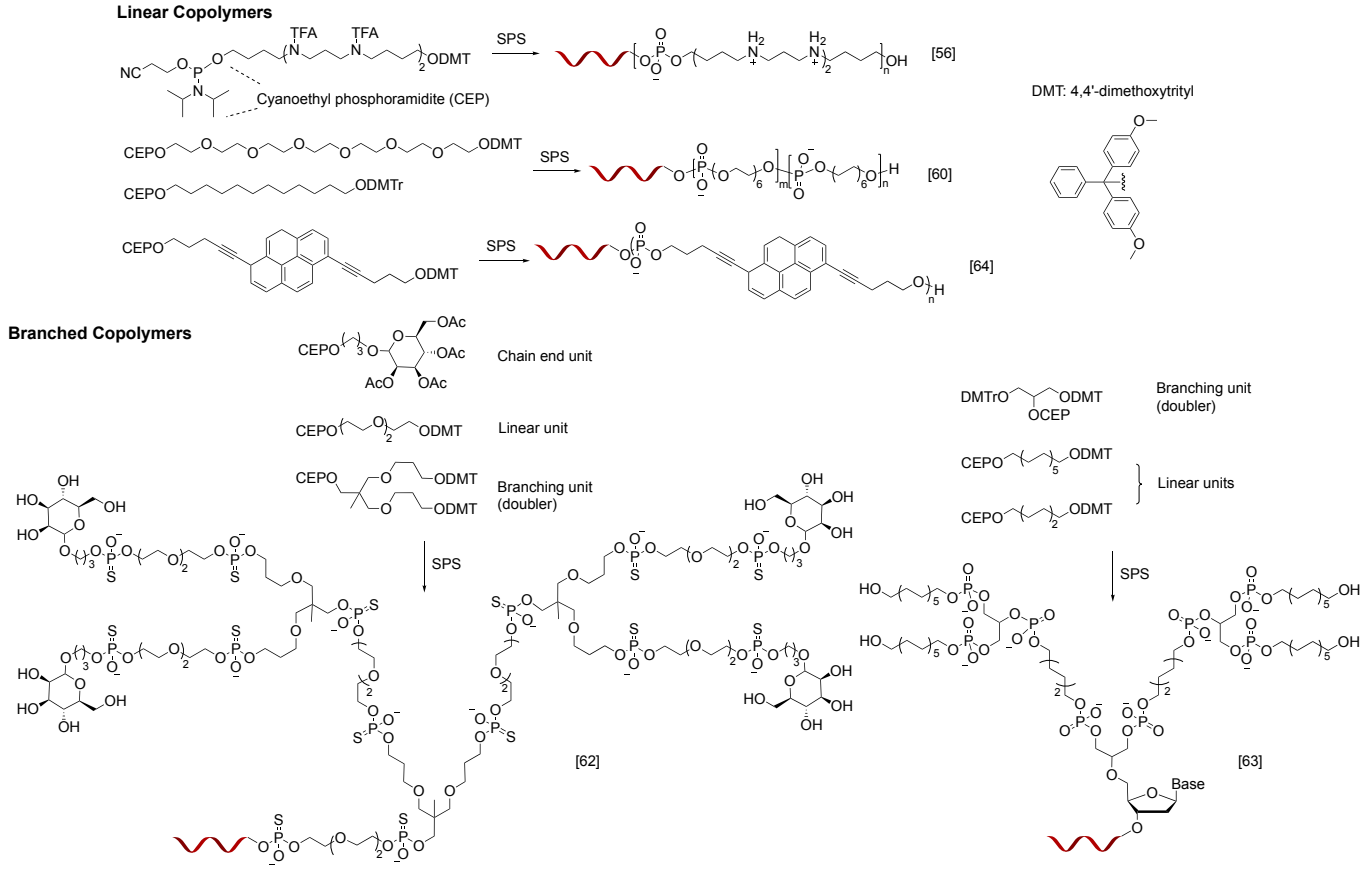


Figure 2. Examples of linear and branched nucleic acid-polymer conjugates prepared via solid-phase synthesis.

For instance, Behr and coworkers reported a series of oligonucleotide-*b*-oligospermine diblock copolymers, termed zip nucleic acids (ZNAs). These structures are produced by introducing varying numbers (1-6) of trifluoroacetyl-protected spermine phosphoramidite monomers during DNA synthesis, providing the conjugate with a cationic tail after deprotection. Spermines are outside edge binders, adding an ionic contribution to the overall stability of DNA duplexes (in addition to Watson-Crick base pairing). This contribution massively increases the duplex melting temperature (ΔT_m as high as 15 °C), which is the function of the number of spermine residues (Figure 2).[56] Later, Corey et al synthesized ZNAs using either DNA or locked nucleic acid (LNA) as antisense agents to target human huntingtin (HTT) and human progesterone receptor (PR). The spermine segment serves to enhance intracellular delivery and target binding, leading to carrier-free, high-efficacy transfection.[57] Similarly, Remy and Kotera et al synthesized di- and triblock siRNA-oligospermine conjugates to target the cell-constitutive natural lamin A/C gene. Interesting, a more potent activity was observed when 30 spermine residues are attached singly to the 5' of the sense strand (diblock), compared to having 15 residues at each of the termini (triblock).[58]

More recently, with an emerging interest in studying the organization of oligonucleotides via hybridization, hydrophobic self-assembly, or both,[59] SPS is being utilized to access well-defined, amphiphilic oligonucleotide-polymer conjugates. Selman *et al.* reported several monodisperse, sequence/length-controlled DNA-*b*-polymer amphiphiles, where hexaethylene (HE) and hexaehtylene glycol (HEG) units were sequentially introduced to the oligonucleotide during DNA synthesis (Figure 2). When 12 HE/HEG monomer units were added onto a 19-mer oligonucleotide, the combined 31 coupling steps resulted in isolated yields ranging from 19 to 29%. These novel materials add a new dimension to the field of DNA nanotechnology by providing a secondary interaction that can be used to organize the nanostructures, encapsulate small molecules, etc (Figure 2).[60] Subsequently, the same group expanded the monomer scope using a variety of phosphoramidites bearing a tertiary amine core. Two substituents of the tertiary amine are used for SPS coupling, while the third substituent carries a desired functionality. Using this method, monomers containing β-D-glucose, alkyne, carboxylate, and phenylalanine derivatives have been successfully added onto oligonucleotides.[61]

In addition to linear structures, conjugates involving dendritic polymers have also been successfully synthesized via SPS. Fréchet et al. reported G1 and G2 dendrimers bearing mannosylated chain ends and an oligonucleotide focal point (Figure 2). In order to create the branching units, "doubler" and "tripler" phosphoramidites were used, which bears two and three dimethoxytrityl-capped hydroxyl groups, respectively. Upon deprotection, each of the free nucleophilic sites can lead to chain extension. The glycodendron with four mannoses conjugated to a thiolated 21-mer oligonucleotide exhibit a precise measured mass of 10575.7 Da.[62] Sleiman et al recently reported an analogous G2 dendrimer (Figure 2), but with a hydrophobic, alkylated dendron. Remarkably, after the conjugate is hybridized to the eight corners of a cube-shaped DNA nanoscaffold, the dendrons fold into the cube to interact with each other via hydrophobic interactions, forming essentially a cube-shaped micelle.[63]

Other than fully covalent oligonucleotide-polymer conjugates, SPS-based methods also provide access to a class of supramolecular polymers, where a DNA-ligand system is used as a monomer for crystallization into higher-order structures. For instance, Häner et al reported an amphiphilic chimeric pyrene-DNA oligomer (Figure 2), which, when immersed in an aqueous medium, can assemble into a helical ribbon supramolecular polymer reaching up to several hundred nanometers in length. The number of pyrene units in conjugate is critical for the formation of the elongated supramolecular polymer; while seven pyrenes per conjugate yielded the assembly, having four or less did not.[64] In these supramolecular polymers, the DNA component remains able to hybridize to the complementary strand.[65] When two supramolecular polymers bearing complementary DNA sequences are mixed, network formation was observed. Upon thermal disassembly and reannealing, the initial suprapolymer blend is converted to a supramolecular random copolymer, losing the ability to aggregate.[66]

Nucleoside/Nucleobase-Functionalized Polymers

While SPS provides unparalleled control over the structure of the nucleic acid-containing copolymer, it is limited by the accessible architectures as well as scale of synthesis. A strategy that in principle can solve these problems involves nucleobase-functionalized synthetic polymers. Unlike DNA and RNA, the backbone of nucleobase-functionalized polymers usually consists of enzymatically and hydrolytically stable bonds such as carbon-carbon bonds, amides, thioethers, etc. The backbone is often sufficiently flexible to accommodate the geometry requirements for base stacking. Using living polymerization techniques, sequential click chemistry, and post-polymerization modification, nucleoside/nucleobase-functionalized polymers can be prepared easily and in large quantities, and a variety of architectures can be achieved. In addition, the nucleobase can be expanded significantly beyond the four-letter library associated with natural nucleic acids. However, a severe downside is the difficulty in controlling base sequence precisely and oftentimes limited solubility in aqueous solutions. Here, we focus on the various polymerization chemistries used; for more detailed reading and potential applications, there is a recent review on this topic by Tang and Zan.[67]

As early as the 1960s, T'so and Takemoto independently reported the first synthetic nucleic acid analogs, which were achieved via free radical polymerization using nucleobases containing *N*-vinyl derivatives.[68, 69] However, only homopolymers or random copolymers with poor polydispersity

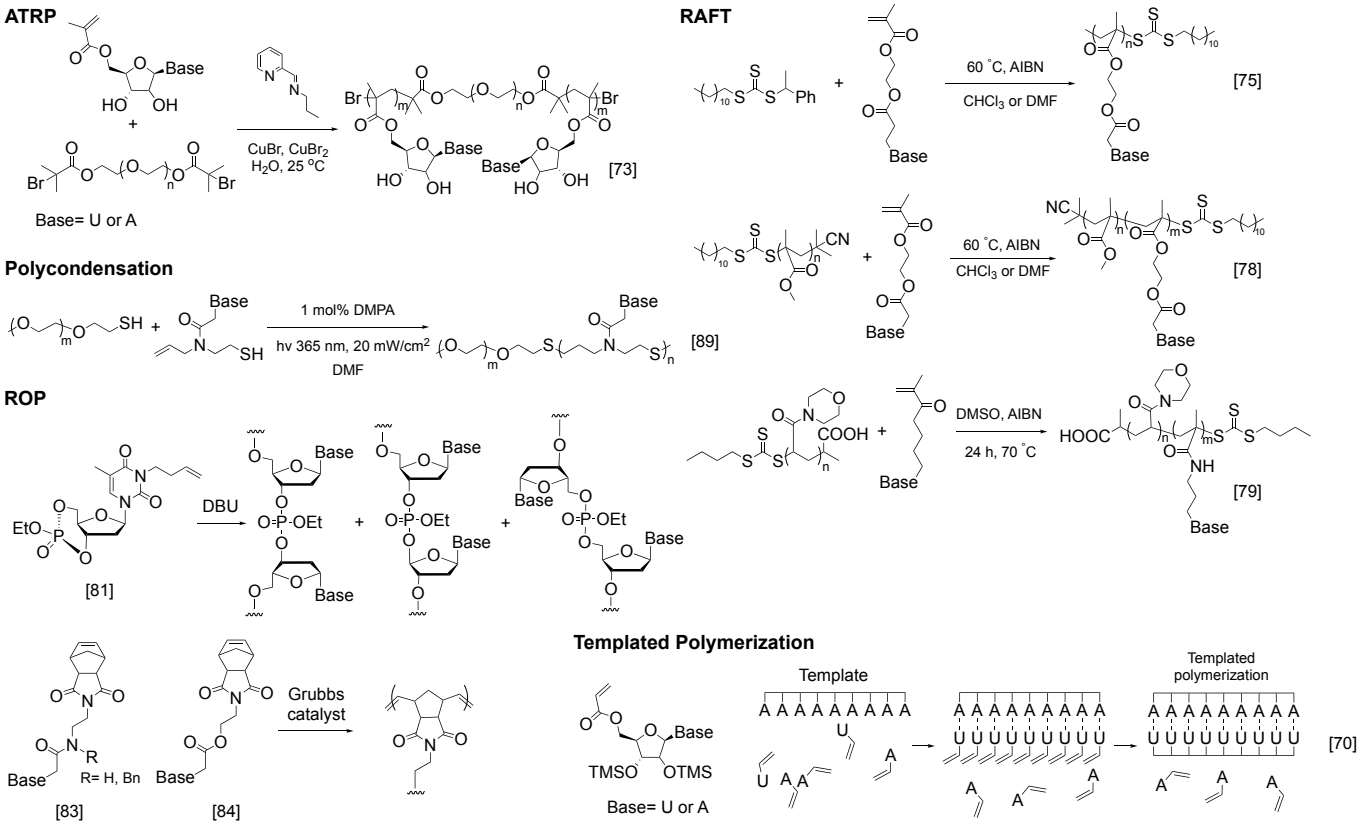


Figure 3. Examples of the polymerization strategies to access nucleoside/nucleobase-functionalized polymers

can be synthesized due to the uncontrolled nature of conventional free radical polymerization. This general approach received renewed interest with the emergence of living polymerization. Marsh, Haddleton et al successfully synthesized uridine- and adenosine-functionalized polymers through ATRP in solution and on solid support using nucleoside-substituted methacrylate monomers (Figure 3).[70, 71] Remarkably, poly(5'-acryloyluridine) can act as a template in the radical polymerization of the complementary 5'-acryloyladenosine in the presence of the noncomplementary 5'-acryloyluridine.[72] The work was later expanded to include the synthesis of water-soluble triblock and pentablock poly(methacryloyl nucleosides) by using bi-functional PEG macroinitiators (Figure 3).[73] Van Hest et al adopted a similar strategy in the polymerization of methacryloyl-derivatized monomers bearing all four nucleobases (thymine, adenine, cytosine, and guanine).[74] Interestingly, in the case of cytosine, a stronger copper-binding ligand (*N,N,N',N",N"*-pentamethyldiethylenetriamine) was used to gain control over the polymerization, which implies that the basic nature of the nucleobases may be problematic in reactions with transition metal complexes.

Unlike ATRP, RAFT polymerization does not involve a transition metal complex and may be more compatible with nucleobase-functionalized monomers. O'Reilly et al have extensively investigated nucleobase-conjugated methacrylates for use in RAFT polymerization (Figure 3).[75-79] Several amphiphilic block copolymers consisting of poly(acryloylmorpholine) or PEG as the hydrophilic block and the nucleobase-containing polymer as the hydrophobic block were synthesized. Upon self-assembly in aqueous buffer, the nucleobase functionality forms the core of the micellar nanoparticles. Unexpectedly, upon mixing with a micelle containing the complementary nucleobase copolymer, the nucleobases are still able to interact despite being sequestered in the core, causing particle morphological and/or size changes. The group also explored the assembly of a fully hydrophobic diblock copolymer, poly(methylmethacrylate)-*b*-poly(methacryloylthymine), in organic solvents. Again, in the presence of an adenine-containing mediator, N9-hexyladenine, the morphology and/or size of the assembled particles are different from the assemblies without the mediator, suggesting that the hydrogen bonding interaction is sufficient to offset the reduced base stacking in organic solvents.[78] This principle was used by Long et al in the work on "supramolecular adhesives", where adenine- and thymine-containing polymers forms supramolecular crosslinks in chloroform.[80] The increased importance of hydrogen bonding relative to base stacking is important even at the monomer stage: it was observed that adenine- and thymine-containing monomers pre-associate in chloroform, causing the polymerization to yield alternating copolymers.[75]

Another polymerization technique adapted for building nucleobase-functionalized polymers involves ring-opening polymerization. Wooley et al reported a cyclic nucleotide monomer (3-butenylthymidine 3',5'-cyclic monophosphate triester), which can be polymerized anionically to give a homopolymer with 70% 3'-5' linkages (the rest are other

isomeric forms).[81, 82] These polymers bears high structural similarity to natural DNA, with the exception of the modified thymine and a phosphotriester backbone. Gibson and co-workers first reported the synthesis of norbornene conjugated thymine, adenine, cytosine, and guanine monomers and their polymerization into homopolymers through ROMP (Figure 3).[83, 84] However, a high degree of polymerization was not achieved (DP_n=5-8) due to solubility limitations. Subsequently, Sleiman et al expanded upon Gibson's earlier work to synthesize well-defined, adenine-containing block copolymers and investigated their self-assembly. Similar block copolymers containing either thymine or diamidopyrimidine, a nucleobase analogue, were later used to template the polymerization of monomers with complementary hydrogen bonding characters.[85, 86]

The idea of DNA-templated polymerization was carefully examined by Liu et al. In order to create a higher degree of sequence control, the Liu group designed a four-base PNA monomer, aldehyde-TCAG-amine, which recognizes "codons" (5'-AGTC-3') on a template DNA strand. Reductive amination yields repeats of the four-base PNA sequence with the length determined precisely by the DNA template.[87] The same technique was later used to template the synthesis of synthetic polymers without structural analogy to nucleic acids, which was achieved by linking the "anticodon" region of the monomer (a pentamer PNA) to the polymerizable region via a disulfide linker. Cleavage of the linker post-polymerization yields the final polymer lacking any nucleobases.[88] Of note, these polymers require a step-growth mechanism to assemble, which can be carried out in solution as well, as such Bowman's series of "clickable nucleic acids" synthesized via thiol-ene click reactions (Figure 3).[89, 90]

Conclusions and Outlook

To summarize, we have presented various synthetic strategies for nucleic acid-containing copolymers. Post-polymerization conjugation remains the most flexible approach regarding the types of nucleic acid/polymer that can be used and the accessible architectures of the conjugate. However, when it comes to amphiphilic conjugates and conjugates with a high-density arrangement of nucleic acids, direct involvement of nucleic acid as a macromonomer or initiator for polymerization can achieve better results, although the solubility of nucleic acids in organic solvents is generally poor and requires extra steps to improve. The highest-quality copolymers are synthesized via solid-phase reactions, which incorporate monomers into the polymer in the same fashion as the incorporation of nucleotides, resulting in absolute control over the degree of polymerization, polydispersity, and polymer architecture. However, solid-phase approaches suffer from poor overall yields for structures requiring high coupling numbers, difficulty in synthesizing copolymers with multiple nucleic acid strands, and limited reaction scales. Conversely, it is possible to synthesize large scales of nucleoside- or nucleotide-functionalized polymers using conventional polymerization reactions, where the backbone of the nucleic acid is replaced with a synthetic polymer. With this approach, it is possible to synthesize novel polymers with unusual features such as non-natural nucleobases, enzymatically stable backbone, solubility in organic solvents, and ability to form hydrogen bonding in bulk. A serious downside, however, is the lost ability to control base sequence.

It is clear that each method has its own limitations, which requires delicate balancing according to specific scenarios. However, every challenge is an opportunity. This area of study is advancing rapidly, and limitations may become irrelevant with new techniques. One way to further advance the field would be to integrate different approaches and thereby maximizing their respective advantages. It is also promising to combine efficient and compatible polymerization reactions with organics-soluble, protected forms of nucleic acid to expand the materials space of the latter.

Author Contributions

All authors contributed to the writing of the manuscript. H. L. and J. C. contributed equally.

Conflicts of Interest

There are no conflicts to declare.

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