Trends in Chemistry

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Review

Transforming bio-derived DNA into biotechnology

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DNA technology is rapidly expanding, with recent advances pushing functional DNA-based materials toward larger scales. Yet producing chemically modified DNA beyond the milligram-scale remains prohibitively expensive, challenging, and relatively unexplored, limiting industrial and translational use. This review article highlights emerging strategies for sourcing, modifying, and purifying DNA at scales relevant for materials and biotechnology. We compare bio-derived DNA sources (e.g., phage, plasmid, and genomic DNA) to conventional synthetic methods and examine their trade-offs. Critically, we re-examine recent and classic literature to identify chemical and enzymatic reactions practical for modifying bio-derived nucleic acids at relevant scales. Finally, we discuss scalable purification and characterization methods to support high-throughput workflows, enabling broader use of bio-derived dsDNA in next-generation applications.

Highlights

Solid-phase oligonucleotide synthesis (SPOS) and rolling circle amplification (RCA) provide precise sequence definition and modifications but remain cost- and waste-intensive, reducing practicality beyond gram-scales.

Bio-derived DNA sources such as phage, plasmid, and genomic DNA offer scalable, low-cost alternatives but require trade-offs in sequence control, purity, and dispersity.

Post-synthetic modification of dsDNA faces challenges due to limited reactive sites, narrow reaction conditions, and viscosity issues at high concentrations.

dsDNA: from genetic information to structural polymer

Deoxyribonucleic acid (DNA)-based materials and biotechnology are increasingly being applied to extend DNA beyond its evolved role as genetic information. By leveraging both the sequence-defined programmability and the polymeric nature of DNA researchers have unlocked a wide range of applications including structural scaffolding such as DNA origami [1], aptamer-based sensors [2], DNA nanocarriers [3,4], DNAzymes [5,6], molecular computing [7], DNA nanowires [6,8], energy conversion systems [9], and synthetic biology platforms [8,10]. A common thread across these diverse uses is the need for precise sequence control to encode function coupled with the incorporation of non-native moieties to achieve desired properties and function. The introduction of such modifications addresses several critical needs which include enhancing chemical or enzymatic stability through backbone modifications, introducing new binding motifs for molecular recognition, enabling conjugation to proteins, nanoparticles, or other macromolecules, improving cellular uptake and biodistribution through lipophilic or targeting groups [11], tuning physicochemical properties such as hydrophobicity or rigidity [12], and enhancing catalytic capabilities by installing cofactors [13]. Thus, the expansion of DNA nanotechnology is intimately tied to both sequence programmability and the introduction of chemical diversity. Unfortunately, translation to clinical and industrial applications is profoundly restricted as the synthetic and enzymatic methods used for production are constrained to the picogram–milligram range due to high cost and lack of methodologies to modify, handle, and purify DNA nanotechnology precursors on the gram and kilogram scale [14,15].

In this review, we explore strategies to obtain inexpensive sources of single stranded (ss) and double-stranded (ds) DNA from biological or bio-derived sources and investigate methods amenable to transform them into functionalized DNA biotechnology precursors on the gram scale. We organize our discussion into three key areas: (i) common methods used to produce dsDNA and their viability at scale, (ii) chemical and enzymatic modifications of dsDNA which have potential to scale, and (iii) practical considerations for handling, mixing, purifying, and

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characterizing large batches of dsDNA. Throughout, we additionally prioritize accessible methods which avoid expensive reagents or specialized infrastructure. While there is no singular method which can currently be leveraged to solve scalable DNA production, this review should allow scientists to pursue alternate dsDNA sourcing, modular functionalization, and practical bulk handling strategies to transition DNA nanotechnology into macroscale applications moving beyond current synthetic constraints.

DNA sources: solid-phase oligonucleotide synthesis (SPOS), biosynthetic, phage, genomic, and plasmid DNA (pDNA)

Achieving gram-scale production of dsDNA requires careful consideration of sourcing methods, each with distinct advantages and limitations in terms of scalability, cost, purity, and sequence flexibility (Table 1 and Figure 1). Currently, DNA materials rely heavily on **SPOS** (see Glossary) (Figure 1A), which enables the production of sequence-defined oligonucleotides with site-specific modifications to the phosphate backbone, ribose sugar, and nucleobase (Figure 1A). While SPOS has truly spawned the field of DNA biotechnology, it suffers significantly from poor economy of scale. SPOS requires large amounts of anhydrous organic solvents, reagents, and consumables which drive up costs and generate significant amounts of chemical waste. Additionally, SPOS faces limitations including inefficient incorporation of modified nucleotides, restricted maximum lengths, and degradation during synthesis and handling [16]. These challenges become more pronounced with longer sequences and larger batches, which compounds the need for alternate approaches. Solution phase approaches, which avoid the low loading capacities of resins, have been used to make gram-scale amounts of DNA polymer conjugates, but lack sequence specificity [17].

Cell-free amplification technologies, such as **rolling circle amplification (RCA)**, offer excellent sequence fidelity, incorporation of non-native nucleotides, high purity, and complete control over sequence design, making them highly attractive for DNA nanotechnology (Figure 1B) [18–20]. However, RCA is constrained by low yields, template instability [19], and the need for extensive purification to remove proteins, salts, and byproducts [21]. It is also highly sensitive to contamination, including nucleases and endotoxins [22]. Additionally, **deoxynucleotide triphosphate (dNTP)** analogs are challenging to access and have reduced ability to be modified [23]. Single-batch production is often limited by enzyme activity and reaction kinetics, however, emerging strategies, including cell-free extract systems and continuous-flow amplification reactors, have begun to push these technologies closer to true gram-scale production at more competitive costs [24]. As RCA matures, cell-free amplification has potential to bridge the gap between the precision of synthetic DNA assembly and the scalability of biological systems.

Aside from RCA, there are many additional enzymatic methods which have been widely used to produce DNA nanotechnology elements. These include **primer extension (PEX)**, **polymerase chain reaction (PCR)**, **nicking enzyme amplification reactions (NEAR)** [14,25], **recombinase-polymerase amplification (RPA)** [26], and template-free enzymatic DNA **sequencing by synthesis (SBS)** such as RCA coupled with TdT [27] (Table 1). Incorporation of a wide range of modified dNTPs has been demonstrated using these biosynthetic methods. Of the previously listed methods, PEX shows the most promise for scalability as successful synthesis of milligram quantities of base-modified oligonucleotides has been reported [28]. While advancements in parallelization and flow reactors could enable higher throughput, similar to RCA, these sources are currently limited to sub-milligram-scale due to expense of enzymes, small windows of viable reaction concentrations, and use of SPOS-derived primers. We encourage the reader to explore comprehensive reviews on these techniques for a more in-depth analysis [14,27,29].

Glossary

Agarose gel electrophoresis (AGE): a rapid, inexpensive technique that separates DNA or RNA based on size and topology.
Blunt ends: ends of linear DNA which have no overhanging nucleotides.
Deoxynucleotide triphosphate (dNTP): the fundamental building blocks of DNA, containing a deoxyribose sugar, a triphosphate group, and one of four nitrogenous bases (adenine, cytosine, thymine, or guanine).
Downstream processing: processes at the end of the DNA production pipeline, such as purification.
Genomic DNA: sheared chromosomal DNA isolated from biological tissues such as salmon milt and calf thymus.
Hydrogel: a three-dimensional hydrophilic crosslinked network infiltrated with a high water content.
Intercalator: planar molecules that fit between base pairs in dsDNA, often affecting its structure and properties.
Major groove: the wider of the two grooves formed by the double helix structure of dsDNA.
Nicking enzyme amplification reaction (NEAR): an isothermal DNA amplification technique that utilizes DNA polymerase and nicking enzyme to produce copies of a DNA sequence.
Phage DNA: DNA found within bacteriophages, viruses that infect bacteria.
Plasmid DNA (pDNA): circular dsDNA produced by bacteria which is independently replicated from genomic DNA.
Polymerase chain reaction (PCR): a technique that utilizes enzymes and thermal cycling to rapidly amplify DNA sequences.
Primer extension (PEX): a technique whereby reverse transcriptase is utilized to extend a DNA primer along an RNA template to generate a complementary strand of DNA.
Recombinase-polymerase amplification (RPA): an isothermal technique similar to PCR that utilizes enzymes, including recombinase, to amplify DNA sequences.
Rolling circle amplification (RCA): continuous replication of a circular ssDNA template to produce large quantities of ssDNA with a repetitive sequence.
Sequencing by synthesis (SBS): a technique that elucidates the sequence of DNA via sequential addition of labeled

Phage DNA amplification from *Escherichia coli* (*E. coli*) is another attractive source for DNA nanotechnology especially in the context of DNA origami [30–32]. Phagemids, such as M13 or T7 in combination with a helper plasmid, can yield up to 590 mg of ssDNA per liter of culture at a cost of $27 per mg (Figure 1C) [31,33,34]. While phage genomes can be engineered, full sequence customization remains limited, and the resulting ssDNA typically requires additional purification to reach sufficient quality, with protein and endotoxin removal complicating **downstream processing** and increasing costs [30,32,35,36]. Double-stranded phage DNA is commercially available and has been used to investigate fundamental polymer physics relationships but is prohibitively expensive for macroscale synthesis[i] [37].

Genomic DNA extracted from biomass provides another high-yielding option, notable for its low cost and straightforward scalability (Figure 1D). Large-scale genomic DNA extraction using tissue homogenization, sodium chloride extraction, and precipitation can produce hundreds of milligrams of DNA, with costs as low as $0.14 per mg, [38]. For example, genomic DNA has been used as a filler or scaffolding material in **hydrogels** and composites [39,40]. However, its utility is confined to applications where only bulk DNA mass is required, and sequence-defined functionality is not essential as there is no control over sequence composition. Due to its heterogeneity, mixture of both single- and double-stranded regions, and presence of contaminants like proteins, lipids, and RNA, genomic DNA would require extensive processing to be useful in precise biotechnology applications [39–41].

pDNA production using *E. coli* offers a combination of sequence customizability, high purity, and scalable yields (Figure 1E). While small-scale laboratory systems typically yield 10–30 mg of pDNA per liter of culture, with costs around $2.70 per mg, industrial bioreactor systems, optimized through advances in **upstream processing** such as fermentation control, feed strategies, and media composition, can achieve yields exceeding 2.6 g of pDNA per liter of culture [42–44]. These large-scale

nucleotides to DNA fragments adhered to a solid support.
Solid-phase oligonucleotide synthesis (SPOS): method for generation of specific oligonucleotide sequences utilizing a solid support and phosphoramidite chemistry for synthesis.
Sticky ends: ends of linear dsDNA with overhanging nucleotides on either strand.
Tangential flow filtration (TFF): purification technique that relies on flow parallel to the membrane instead of through it to reduce stress on biomolecules such as DNA.
Upstream processing: processes at the beginning of the DNA production pipeline, such as cell culturing.

Table 1. Comparison of common sources for DNA materials

	SPOS	RCA	PEX	PCR	NEAR	RPA	SBS	Phage	Genomic	**pDNA**
Length	<100 nt	[a]	8–31 bp	<10 kbp	10–22 nt	100–200 bp	<1000 nt	3.2 to >200 kb	0.1–40 kb	1 – 115 kb
ssDNA/dsDNA	ssDNA	ssDNA	dsDNA	dsDNA	ssDNA	dsDNA	ssDNA	ssDNA or dsDNA	Mixture	dsDNA
Dispersity	Uniform	Variable	Uniform	Low	Uniform	Low	Uniform	Uniform	High	Uniform
Functionality	+++	+	++	+	+++	+	+++	Native	Native	Native
Sequence control	++++	+++	+++	+++	+++	+++	++++	+	–	++
Source	Synthetic	Enzymatic	Enzymatic	Enzymatic	Enzymatic	Enzymatic	Enzymatic	Bacteriophage	Salmon milt Calf thymus	Bacteria
Scale	< mg	mg	mg	µg	nM	nM	µM	mg	g	g
Cost	$0.15/nt[b]	$379/mg	[c]	[c]	[c]	[c]	[c]	$27/mg	$0.14/mg	$1.4/mg
Green	No	No	No	No	No	No	No	Yes	Yes	Yes
Refs	[16,17,55]	[19,20,47]	[28]	[14,25]	[14,25]	[26]	[27]	[1,37]	[38,40,41]	45

[a]Length of RCA derived DNA varies widely but is often hundreds of thousands of nucleotides. Costs are determined from either literature reports or calculated from commercially available sources.
[b]Cost of SPOS-derived DNA is heavily dependent on sequence length and purity.
[c]Cost of these biosynthetic sources is unknown because they have not been scaled as traditional applications are for sequencing or diagnostic purposes. Cost is expected to be much higher than other methods reported in this table due to the use of one or more enzymes and low yields.

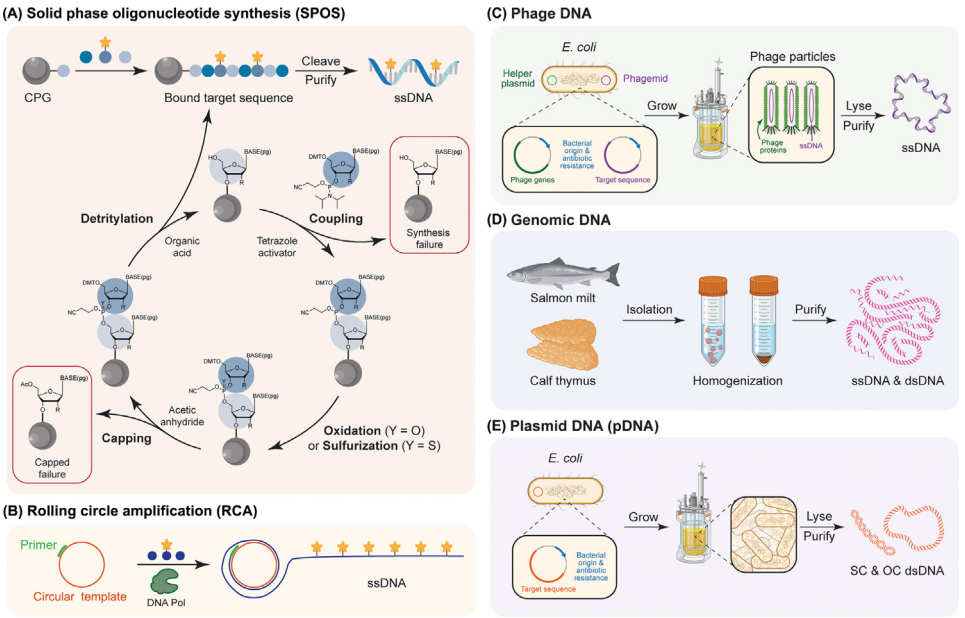

Figure 1. Production of different sources of DNA for materials. (A) Single-stranded DNA (ssDNA) made through solid phase oligonucleotide synthesis (SPOS). Target sequence is synthesized on the surface of a controlled pore glass (CPG) solid support using phosphoramidite chemistry. Oligonucleotides are cleaved/deprotected and full-length sequences are purified from off target sequences using chromatography. (B) Rolling circle amplification (RCA). Single-stranded circular template with primer is amplified using DNA polymerase to create long sequences of ssDNA. (C) Phage DNA is made by introducing a phagemid containing the target sequence and a helper plasmid that codes for phage genes into *Escherichia coli* (*E. coli*). During bacterial growth in a bioreactor, phage particles are made by encapsulating the circular ssDNA in phage proteins. Following lysis, target single-stranded phage DNA is then purified to yield uniform samples of circular ssDNA. (D) Genomic DNA is isolated from the tissue of a biological source (e.g., salmon milt or calf thymus). Following tissue homogenization, the genomic DNA is purified using phenol/chloroform extraction to yield a disperse DNA sample that contains ssDNA and double-stranded DNA (dsDNA). (E) *E. coli* containing the target plasmid are grown in bioreactor. Cells are lysed using alkaline lysis and target plasmid DNA (pDNA) is purified using anion exchange chromatography to yield uniform samples that contain supercoiled (SC) and open circle (OC) isoforms of dsDNA. For (A) and (B), modified nucleotides can be incorporated to introduce non-native functionality (orange star). Modified nucleotides cannot be introduced into sequences made using (C–E). Abbreviations: Base(pg), nucleobase with protecting group; DMT, dimethoxytrityl.

systems employ alkaline lysis followed by anion exchange chromatography for downstream processing, building on foundational protocols refined for high-capacity, pharmaceutical-grade output [43,44]. To extend the utility of these advances to academic settings, our group developed a benchtop-compatible workflow that miniaturizes key industrial steps, enabling routine production of 1–2-g batches from 2–5-l cultures in standard lab fermenters at a reduced cost of $1.40 per mg. This streamlined process retains the high purity and sequence flexibility of industrial systems while making scalable pDNA production accessible to non-industrial laboratories [45].

The aforementioned methods highlight the trade-off between low cost and scalable techniques with sequence control and reliable incorporation of modified nucleotides. While this list is not meant to be exhaustive of all types of DNA production and their analogs, we hope it guides the reader to potential new sources of oligonucleotides.

Scalable chemistry for bulk DNA modification

A limitation in many of the aforementioned scalable sources of DNA is the inherent lack of non-native functionalities which are essential for many nanotechnology and biotechnology

applications. Thus, it becomes important to investigate methods which could modify bulk bio-derived sources into useful precursors for DNA biotechnology. Common modification sites include backbone substitutions, internal modifications through non-standard bases, and functional groups at the 5′ and 3′ termini (Figure 2A). Traditionally, the most robust way to incorporate these modifications is to use SPOS and modified phosphoramidites. However, the desired functionality must be tolerant of SPOS chemistry and many modified phosphoramidites are not commercially available and are synthetically cumbersome to access [46]. Therefore, reactive functional handles such as amines, thiols, alkynes, and azides are often incorporated to couple desired functionality post-synthesis. Common targets for constructing DNA materials include fluorophores, proteins, polymers, redox labels, catalysts, and crosslinkers (Figure 2B) [14,29]. Applications of these

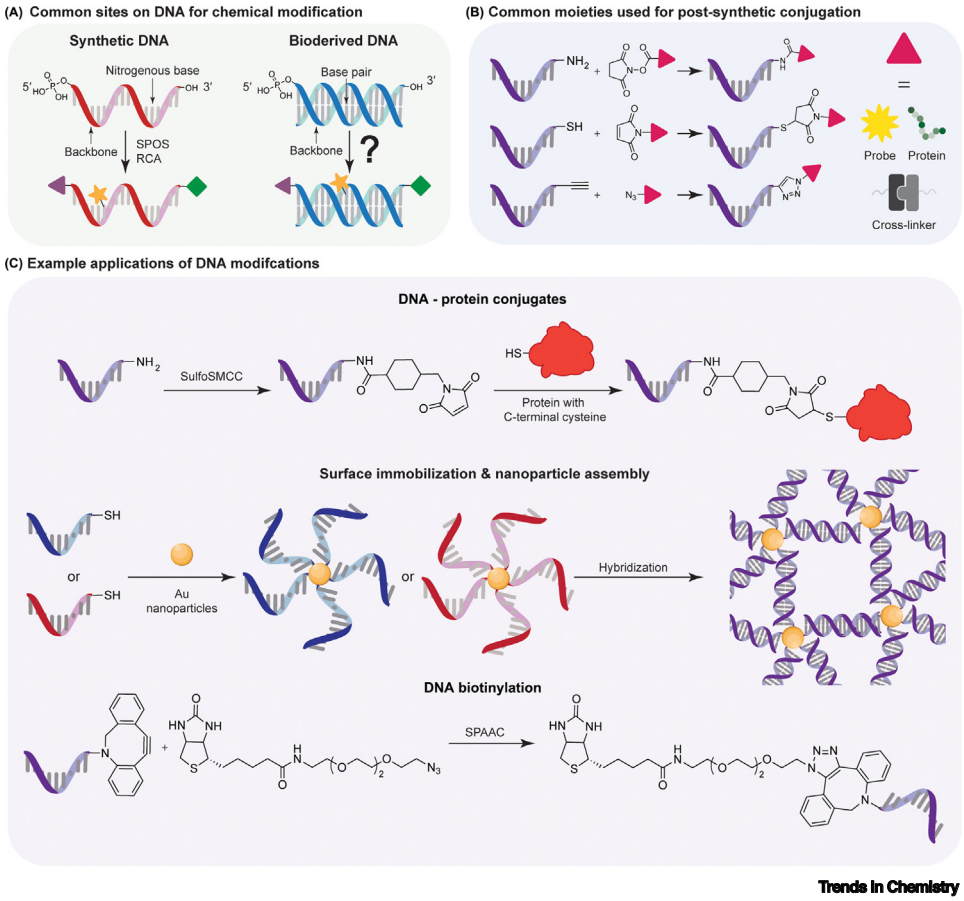

Figure 2. Current approaches for generating modified oligonucleotides. (A) Cartoon depiction of common sites for modification of DNA. Synthetic methods (e.g., SPOS, RCA, etc.) are commonly used but do not translate to modifications of bio-derived dsDNA. A wide variety of moieties can be incorporated if they are compatible with reaction conditions and are outfitted with a phosphoramidite group. Desired functionality can be directly incorporated during synthesis or conjugated in subsequent reactions. (B) Common functional handles incorporated into DNA for post-synthetic modification and their corresponding reactive partner. From top to bottom: amines and ***N***-hydroxysuccinamide, thiols and maleimides, azides and alkynes. Functionality is dependent on specific applications but are often polymers, fluorescent probes, proteins, or crosslinkers. (C) Example applications of post-synthesis modifications. From top to bottom: amino-modified DNA to produce DNA–protein conjugates using the heterobifunctional crosslinker sulfosuccinimidyl 4-(***N***-maleimidomethyl)cyclohexane-1-carboxylate (sulfoSMCC), thiol-modified DNA to immobilize sequences onto gold surfaces or nanoparticles. Introduction of complementary strands allows for self-assembly via hybridization. Dibenzocyclooctyne-modified DNA for biotinylation of DNA via SPAAC.

handles include coupling of amino-modified DNA with proteins containing a C-terminal cysteine using a heterobifunctional crosslinker such as sulfosuccinimidyl 4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate (sulfoSMCC), surface immobilization and self-assembly of DNA gold nanoparticles using thiol-modified DNA, and dibenzocyclooctyne-modifed DNA for biotinylation of DNA via SPAAC (Figure 2C) [46]. For more exhaustive reviews on common DNA modifications and their subsequent applications we direct the readers to the following reviews [13,18,29,46–48]. While these methods have been used to generate customized materials with advanced functionality and a wide breadth of applications, their utility is diminished in the context of modifying bio-derived DNA. Therefore, investigation of alternate approaches is needed to transform bulk bio-derived dsDNA into functionalized material precursors.

Functionalization of native bio-derived dsDNA presents formidable challenges, especially at larger scales. Traditional chemical and enzymatic modifications are typically carried out at low concentrations and small reaction volumes to maintain regioselectivity and conserve costly reagents. As applications scale from proof-of-concept to real-world deployment, the economics of functionalizing DNA at gram-scale becomes a critical bottleneck [49]. Despite its ubiquity, dsDNA is remarkably inert, with only a handful of weak nucleophilic sites available for modification. These are primarily the 5′ phosphate, 3′ hydroxyl, and the endocyclic amines of the purine bases [50]. Although other potential nucleophilic sites exist along the strand, their low reactivity often necessitates strong electrophiles, which can risk damaging the DNA backbone or disrupting base pairing [51,52]. Moreover, DNA's sensitivity to pH, ionic strength, and temperature significantly narrows its operational window and places further constraints on the range of chemistries that can be used effectively. Evaluating both biological and chemical strategies for efficient DNA modifications that preserves integrity (e.g., base pairing, structure, and sequence) is essential for developing a viable route for scalable production [53]. This section will focus on methodologies that could offer facile and cost-effective solutions for chemical modifications of DNA to produce bulk materials.

Starting at the 5′ and 3′ ends of DNA, there are several alternative strategies that offer scalability or are promising candidates for large-scale implementation [15]. Activation of the 5′ phosphate using carbodiimides and imidazole enables conjugation with primary amines to form phosphoramidate linkages and both reagents are commonly used and employed at the kilogram-scale (Figure 3A). However, this approach can also activate backbone phosphates, resulting in complex product mixtures with off-target labeling, though in some cases such side reactions may still prove useful [54].

Alternatively, modified dNTPs can be incorporated enzymatically (Figure 3B) with terminal deoxynucleotidyl transferases (TdTs) enabling selective 3′ end modification of **blunt ends** or **sticky ends** with 3′ overhangs. Additionally, polymerases can be used to blunt sticky ends with 5′ overhangs while 3′ overhangs result in exonuclease activity [55–57]. Polymerases and TdT accept a wide range of modified dNTPs allowing controlled functionalization of DNA (Figure 3C). Modifications can be introduced at the sugar, phosphate, or nucleobase but base modifications tend to be more broadly tolerated. Furthermore, nucleobase modifications are preferred at the C5 and C6 positions of pyrimidines and the N7 and C8 positions of purines in order to preserve base pairing [58]. A variety of modified dNTPs are commercially available or synthetically accessible to install click handles, fluorescent probes, redox labels, reactive groups, polymers, and proteins, facilitating the development of a wide range of customized DNA materials [14,29]. A few examples are showcased in Figure 3C but for an extensive survey of modified dNTPs we point readers to the following reviews [14,27,29,58]. As stated earlier, enzymatic modification strategies using DNA polymerase or TdT are limited by the high cost of enzymes and

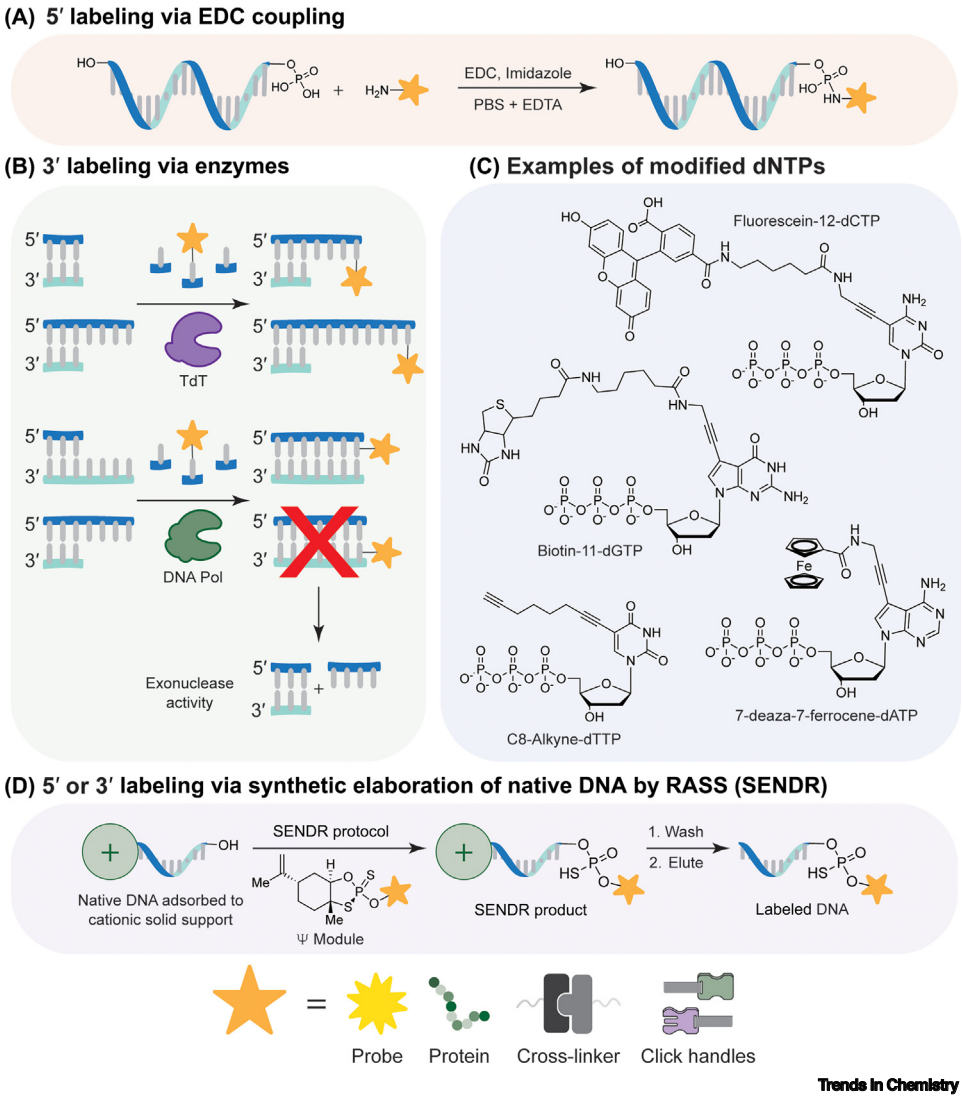

Figure 3. Promising scalable synthetic routes for end labeling of bio-derived DNA. (A) The 5′ labeling of dsDNA via coupling of amines using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and imidazole in PBS buffer supplemented with ethylenediaminetetraacetic acid (EDTA). (B) Common routes for enzymatic incorporation of modified nucleotides onto the 3′ end of dsDNA. Terminal deoxynucleotidyl transferase (TdT) is used for incorporation of deoxynucleotide triphosphates (dNTPs) onto blunted or sticky ends with 3′ overhangs. DNA polymerase is used for incorporation of dNTPs onto 5′ overhangs. Enzymatic incorporation onto 5′ end is not possible enzymatically. Attempts either result in no change to DNA or exonuclease activity where overhangs are removed. (C) Examples of modified dNTPs. (D) The 5′ or 3′ end labeling of DNA using synthetic elaboration of native DNA by reversible adsorption to solid support (RASS) (SENDR) with Ψ modules. A variety of probes, proteins, crosslinkers, click handles, etc. can be incorporated, allowing for end labeling or crosslinking of double-stranded DNA (dsDNA) chains.

custom dNTPs. To increase scalability, it will be important to address these economic barriers through approaches such as improving enzyme efficiency, developing nucleotide recycling strategies, or optimizing reaction conditions [27,56,59,60].

More recently, pentavalent phosphorus [P(V)] reagents have gained attention for their ability to eliminate the oxidation step in SPOS and to control the stereochemistry of phosphorothioate

linkages, which are widely used to enhance nuclease resistance [61]. This platform was further extended using the SENDR method [(synthetic elaboration of native DNA by RASS (reversible adsorption to solid support)], which employs P(V) reagents to selectively functionalize either the 3′ or 5′ hydroxyl groups of dsDNA [54,61]. Due to the low cost and the simplicity of the process, SENDR holds strong potential for scalable, selective DNA labeling. However, the method currently relies on adsorbing nucleotides onto a solid support for subsequent reactivity, which presents some limitation when scaling up (Figure 3D). While numerous strategies exist for modifying DNA, we highlight the aforementioned approaches as particularly promising due to their scalability, accessibility, and compatibility with large-scale materials applications utilizing bio-derived DNA.

Next, we consider chemistries for internal or sequence-specific chemical modifications of bio-derived dsDNA. Similar to end labeling, enzymatic methods have demonstrated great promise for introducing internal modifications. The utility of modified dNTPs is seen in applications such as redox labeling [62,63] hemin/G-quadruplex DNAzymes [63], grafted DNA materials [64], and antibody conjugation [65]. However, these methods for internal modifications are still not scalable due to high cost of enzymes. Furthermore, such enzymatic methods for internal modifications of bio-derived dsDNA are not possible as chains are pre-synthesized, preventing enzymatic incorporation of non-native dNTPs.

When working with bio-derived DNA, the introduction of chemical functionality becomes particularly challenging as most chemical modification strategies lack sequence or site selectivity. Nevertheless, direct functionalization can be achieved using electrophilic alkylators that react with nucleophilic sites on the DNA to form stable covalent adducts (Figure 4A). These reagents bypass the need for sequence-specific enzymatic incorporation but require careful control to preserve DNA integrity and ensure reproducibility at scale. Reagents such as nitrogen mustards, nitrosoureas, and triazenes have long been studied for their ability to alkylate DNA, typically requiring an intramolecular rearrangement to form a reactive species in the form of an activated electrophile [50,51]. These compounds are clinically significant due to their mechanisms of action in cancer therapy, where bis-functional alkylation crosslinks DNA strands, disrupting its role as a carrier of genetic information [66]. The milder nitrogen mustards, found in agents like chlorambucil, share a di-halogen ethylamine core, with less nucleophilic internal nitrogen designed to tune reactivity and hydrolytic stability [67,68]. Their reaction with DNA yields adducts that may undergo hydrolysis to form stable formamidopyrimidine lesions or cleavage of the *N*-glycosyl bond, leading to loss of the base and modification [69–72]. Notably, nitrogen mustards preferentially react at 5′-GNC-3′ motifs, targeting the N_7 position of guanine (N_7G), which is the most nucleophilic site in dsDNA due to its electron density and accessibility within the **major groove** [50,51,69,73]. By contrast, more direct alkylating agents such as epoxides and acrylates have been explored in the construction of DNA-based materials [39,74–76]. However, their reactivity is often limited to exocyclic amines on the Watson-Crick face of nucleobases, sites that are occluded in duplex DNA. As such, these electrophiles tend to react only with ssDNA, restricting their applicability in the context of double helical materials.

Importantly, while alkylation is typically viewed as a nonspecific process, it can exhibit mechanistic preferences for certain nucleotides. S_N2-type alkylators favor N_7G, whereas S_N1-type species preferentially react with O_6-guanine (O_6G) and phosphate backbone oxygens (P_O) [50,51]. These inherent selectivities can be harnessed to design more targeted and reproducible modifications in dsDNA-based materials. Building on this foundation, we have demonstrated the viability of using nitrogen mustards to covalently graft polyethylene glycol (PEG) side chains onto topologically defined dsDNA (linear and circular), creating nuclease-resistant DNA bottlebrush

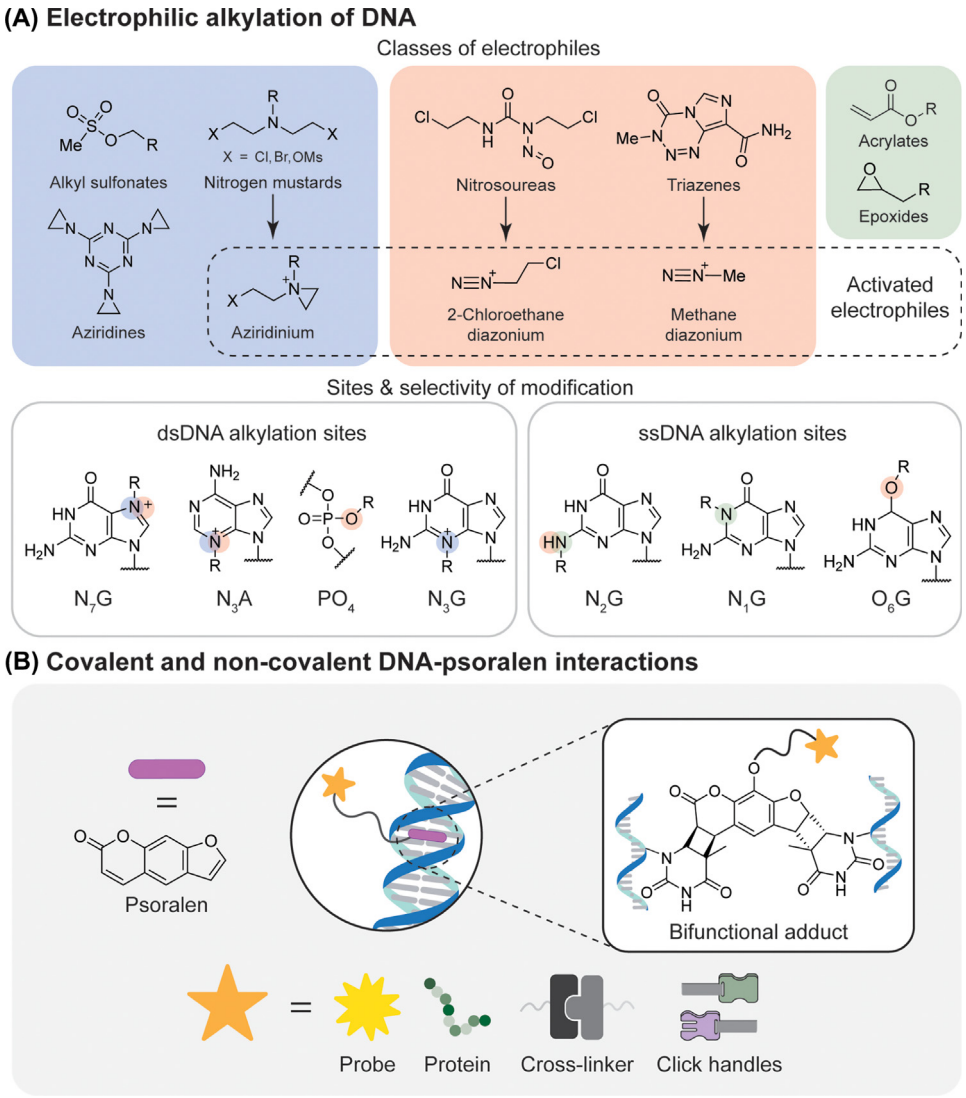

Figure 4. Promising scalable synthetic routes for internal modifications of bio-derived DNA. (A) Electrophilic alkylation of DNA. Common classes of electrophiles are shown with a representative compound. Nitrogen mustards, nitrosoureas, and triazenes form an activated electrophile which is crucial for alkylating activity. Site selectivity of electrophiles is observed based on mechanistic pathway. Blue, S_N2 electrophiles; red, S_N1 electrophiles; green, π–π electrophiles. (B) UV activation of psoralen with double-stranded (dsDNA). Following psoralen intercalation, exposure to UV light triggers a 2+2 cycloaddition with the pyrimidine C5–C6 π system, preferably with thymine bases. Each of these alkylating handles can be conjugated to a variety of probes, proteins, crosslinkers, or click handles allowing for site selective internal modification or crosslinking of dsDNA chains.

polymers suitable for soft matter applications [77]. Given the widespread occurrence of GNC motifs, the synthetic accessibility of nitrogen mustard derivatives, and their robust reactivity, this strategy demonstrates a tenable approach and use for non-specific gram-scale modification of dsDNA for advanced material applications [50,51].

Another promising handle for internal site selective modification of dsDNA is the **intercalator** psoralen (Figure 4B). Photoirradiation of a psoralen-dsDNA intercalation complex results in covalent

adducts with thymines in 5′-TA-3′ and 5′-AT-3′ via 2+2 cycloaddition [78,79]. Psoralen derivatives are already commercially available to tag dsDNA with biotin moieties for biomolecular labeling and the field continues to make strides for more efficient and capable varieties [78]. Beyond the scope of labeling DNA, we envision psoralen-based handles to become a potential avenue for addition of various functional groups as the hydroxy derivative can easily be modified for specific applications. Moreover, psoralen-DNA materials offer promising opportunities in applications such as 3D printing because they can be manipulated on temporal dimensions through photo crosslinks.

By recontextualizing these reactions through the lens of scalability, economic feasibility, and compatibility with bio-derived dsDNA, we aim to highlight a practical roadmap for building high-performance DNA materials using accessible biological feedstocks. Ultimately, this review underscores the need for an integrated approach that aligns synthetic chemistry, biotechnology, and materials science to unlock the full translational utility of DNA.

Handling, purification, characterization of modified dsDNA

Despite the growing number of practical methods to modify DNA on scale, routine reaction conditions, purification techniques, and analysis tools are largely not amenable to DNA chemistry [15]. First and foremost, upscaling conventional DNA-based reactions to the gram level leads to impractically large volumes. For example, a typical TdT labeling reaction might use ~100 ng/μl of dsDNA substrate in a 100 μl reaction volume, along with 5–10 U/μl of TdT enzyme and a tenfold excess of modified dNTP. Scaling this reaction to produce 1 g of modified dsDNA would require at least 10 l of reaction mixture and thousands of units of enzyme, rendering the process unattainable in most academic settings and cost-prohibitive for industrial use due to the high price of enzymes, modified nucleotides, and the large volume of aqueous waste generated [27]. Increasing DNA concentration can reduce reaction volume, but leads to higher viscosity due to polymer entanglement and molecular crowding [15]. This reduces the diffusion rates of reactants and can result in sluggish or incomplete reactions. The extent of this effect is modulated by both the topological state of dsDNA and strand length, while the negatively charged phosphate backbone introduces additional electrostatic barriers that hinder mass transport [80]. Therefore, homogenization strategies are crucial for bulk reactions (Figure 5A).

This presents another major problem as traditional methods of mixing like pipetting, vortexing, or stirring exert high shear forces that can fragment DNA and compromise its function [81,82]. Dual asymmetric centrifugal (DAC) mixers have emerged as an attractive solution, providing efficient homogenization via counter-rotating forces that minimize shear and preserve strand integrity[ii]. Their scalability, from milligram to multi-kilogram, makes them well-suited to the growing demand for large-scale dsDNA modification[ii]. Additional low-shear tools include static mixers, which blend without moving parts, peristaltic or diaphragm pumps for gentle transfer of viscous solutions, and **tangential flow filtration (TFF)**, which concentrates and purifies dsDNA without harsh pressures or interfaces. These techniques are now integrated across the DNA vaccine pipeline, from fermentation and extraction to purification and formulation[iii,iv] [83,84]. Platforms that incorporate these tools for large-scale dsDNA processing with minimal loss and degradation are commercially available[v]. Integrating low-shear infrastructure into scalable DNA biotechnology is essential for preserving the structural integrity of DNA during processing.

Following chemical modification, large-scale isolation of functionalized dsDNA from unreacted reagents and side products is critical (Figure 5B). Desalting columns or spin filters are sufficient for sub-milligram quantities but lack the throughput and capacity required for bulk applications [84]. While unreacted small molecules can be removed using isopropanol precipitation, dialysis, or TFF, the separation of modified sequences will require chromatographic methods [85,86]. A

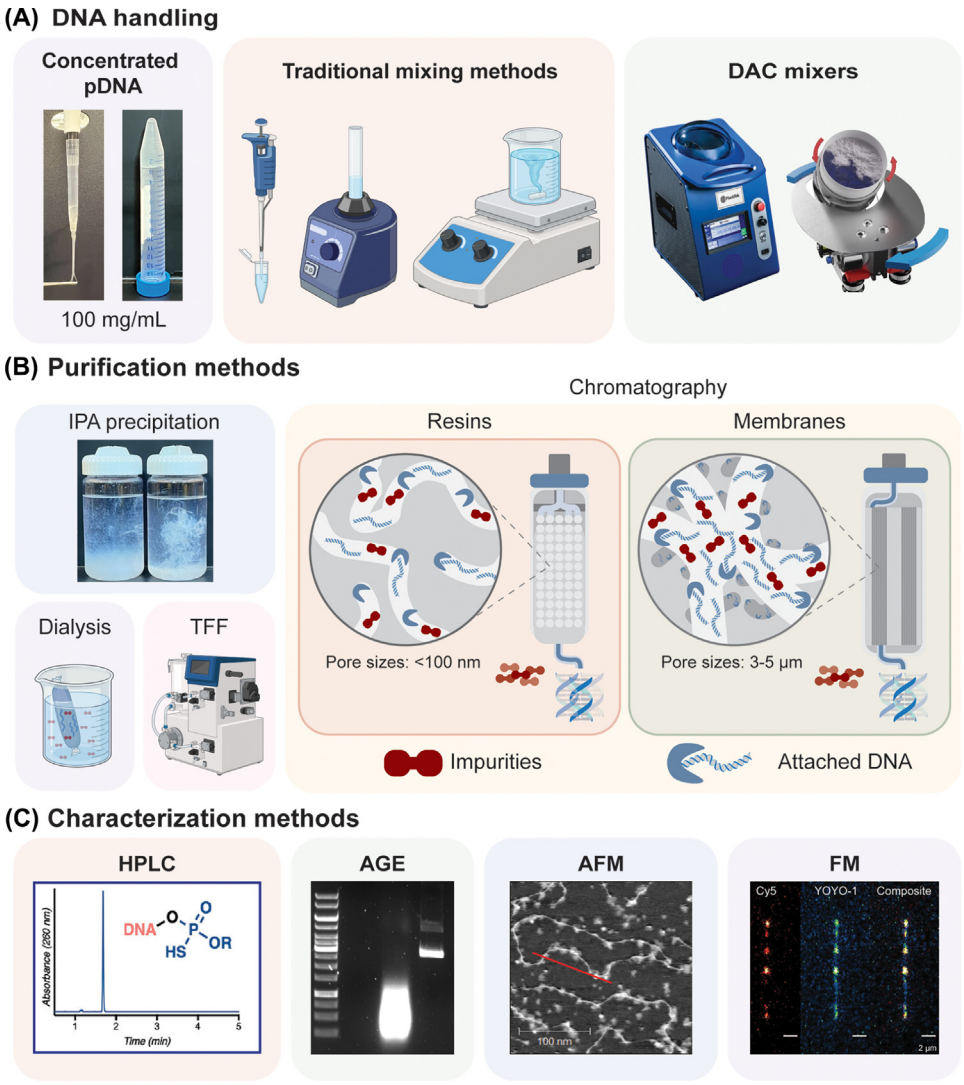

Figure 5. Handling, purification, and characterization strategies for modified DNA. (A) Handling considerations for highly concentrated DNA. Physical entanglements produce weak hydrogels even at low concentrations. Coupled with the sensitivity of DNA to shear induced damage, traditional mixing methods (pipetting, magnetic stirring, and vortexing) are not options. Dual asymmetric centrifugal (DAC) mixers are a promising alternative as they have already been used to homogenize DNA solutions without damage. (B) Purification of modified DNAs. Unreacted small molecules can be removed using isopropanol precipitation, dialysis, or tangential flow filtration (TFF). Separation of functionalized DNAs will require chromatographic separation. Switching to membrane-based chromatography will accommodate the high throughput demand of bulk purifications. Specific chemical modifications will influence selection of appropriate purification. (C) High performance liquid chromatography (HPLC) (reprinted from [54]) is a standard technique used to characterize synthetic oligonucleotides and will likely remain useful in the context of functionalized bioderived double-stranded DNA (dsDNA). DNA is routinely characterized using agarose gel electrophoresis (AGE) but is mainly qualitative. The extreme size and availability of dsDNA-specific dyes lend well to imaging techniques such as atomic force microscopy (AFM) (reproduced from [77] with permission from the Royal Society of Chemistry) and fluorescent microscopy (FM) (reprinted, with permission, from [97]; copyright 2018 American Chemical Society) for characterization of functionalized oligonucleotides. Abbreviation: pDNA, plasmid DNA.

shift away from high performance liquid chromatography (HPLC)-based purification of oligonucleotides has been observed recently due to large volumes of solvent consumption [84,87]. In response, a variety of preparative chromatographic techniques have been adapted for large-scale purification of pDNA and chemically modified nucleic acids for vaccine and gene therapy development [84,88–90]. Among these, anion exchange chromatography remains the most widely adopted strategy due to its robust performance and broad compatibility. Strong anion exchanger resins bearing a quaternary ammonium group are popular choices and are commercially available at an affordable cost, enabling scalable implementation across various production settings [84].

A key limitation of resin-based systems for purification of dsDNA is the orders of magnitude decrease in binding capacity compared with proteins [91,92]. This results in longer processing times, high buffer volumes, and substantial waste generation which hinders scalability and sustainability [88,89,91]. A promising solution can be found in membrane chromatography (MC), as macroporous substrates offer enhanced surface area and convective flow, leading to significantly higher binding capacities and faster flow rates [93,94]. A variety of anion exchanger membranes are commercially available and immediately ready for use, eliminating the need for manual packing, column conditioning, and validation[v,vi]. Additionally, their compact footprint, reduced buffer consumption, operational simplicity, and scalability make them particularly well-suited for the bulk purification of functionalized oligonucleotides.

Although MC presents a promising alternative to resins, a few limitations have been reported during scaling. These include back-mixing, reduced binding capacity with increased column height, lower resolution, and membrane fouling. While potentially substantial, these challenges do not create barriers that prevent scaling of MC altogether. Instead, users must carefully select ligand, pore size, and matrix composition. Ongoing advances in chromatography engineering are poised to significantly expand its utility for high-throughput purification of large biomacromolecules, many of which have historically posed challenges for traditional column chromatography techniques [95].

Beyond mixing and purification, new characterization methods of modified dsDNA will need to be established as conventional methods (LC-MS, NMR, sequencing, etc.) may not be practical due to extreme size of bio-derived dsDNA (Figure 5C). While the practicality of HPLC for preparative purifications diminishes at bulk scale, its utility in an analytical context remains valuable [84,87]. Characterization and analysis of dsDNA is routinely accomplished using **agarose gel electrophoresis (AGE)** and UV-Vis spectroscopy to confirm identity, topology, and purity. AGE can also be used to confirm successful conjugation of modifications which manifests as an increase in the observed molecular weight, but observable changes require extensive modification. This technique is mainly qualitative but degree of modification can be crudely estimated using molecular weight ladders [77]. Fortunately, the unique structure and characteristics of dsDNA makes imaging a convenient alternative for sample characterization. Atomic force microscopy (AFM) is often used to analyze length and topology of DNA but has also been used to confirm attachment of polymer sidechains for synthesizing bottlebrush polymers as well as formation of DNA origamis [1,77,96]. Furthermore, there are a wide range of dsDNA-specific dyes that are available and fluorophores can be incorporated during modification, making fluorescent microscopy (FM) an attractive route for analysis of functionalized DNAs [47,97–99].

Concluding remarks and future perspectives

Over the past two decades, the promise of DNA nanotechnology has steadily expanded, evolving from small-scale structural motifs and proof-of-concept nanosystems to a flourishing toolkit for functional materials, molecular devices, sensors, and biologically responsive scaffolds. The

Outstanding questions

How can enzymatic systems such as TdT and RCA be engineered to produce gram-scale quantities of modified DNA?

What mild, water-compatible chemical reactions can efficiently functionalize dsDNA without compromising base pairing or structural integrity?

What new purification strategies can separate closely related DNA topologies or partially modified products with minimal waste?

How can bulk handling challenges, such as viscosity and entanglement, be mitigated to maintain homogeneity in large-volume reactions?

What scalable analytical methods should be standardized to verify modification levels and quality control for bulk dsDNA?

How can these approaches align with principles of green chemistry to reduce reagent costs, solvent use, and waste generation?

What practical workflows will enable academic and industrial labs to adopt these methods without specialized infrastructure?

Which emerging applications stand to benefit most from overcoming these production and modification constraints?

unique combination of sequence programmability, polymeric properties, and biocompatibility positions dsDNA as an extraordinary building block for new classes of bio-inspired materials. Yet, realizing this vision at scales meaningful for translational applications remains constrained by several interlinked bottlenecks [16].

One of the clearest lessons emerging from this review is that no single DNA source or modification approach is universally sufficient for scaling. SPOS, while unmatched for precision and sequence control, remains economically and environmentally unsustainable for gram-scale production [16]. Meanwhile, cell-free amplification strategies like RCA offer attractive fidelity and design flexibility but remain limited by enzyme cost, yield, and contamination risks [19,22]. Each bio-derived DNA source (phage, pDNA, and genomic), offers unique advantages but also trade-offs in cost, purity, sequence flexibility, and downstream processing [32,39,45].

These realities point to an urgent need for an integrated strategy that combines practical bio-derived DNA production with robust, scalable post-synthetic functionalization. Importantly, achieving this will require revisiting not just the underlying chemistries but also the engineering and process design that translate bench-scale feasibility to industrial practicality. Approaches such as end-group conjugation, SENDR labeling [54], or sequence-selective internal alkylation [50,79] show real promise, yet they are rarely benchmarked at reaction volumes and concentrations that match gram-scale or multi-gram workflows. The narrow operational window of DNA and sparse nucleophilic sites further constrain the suite of reactions that can be used without sacrificing structural integrity, base pairing, or biological performance.

Beyond reaction design, this challenge demands attention to handling, purification, and quality control at scale. The long polymeric nature of bio-derived dsDNA introduces unique constraints: high viscosity, strong intermolecular interactions, and entanglement behaviors make bulk reactions prone to heterogeneity and low yields if not well-mixed [15]. Shear-sensitive mixing tools like DAC mixers[ii], static mixers [83], and membrane-based purification systems [95] offer promising solutions, but their integration into DNA materials pipelines is still emerging. Similarly, high-throughput, scalable analytical methods for verifying degree of modification, sequence integrity, and topology will be critical to support reliable large-batch production.

Another theme that emerges is the need for greener and more sustainable chemistries. Many of the most effective modification strategies rely on large volumes of organic solvents, hazardous electrophiles, or reagents that are cost-prohibitive to deploy at scale. Developing water-compatible, mild, and selective reaction conditions for dsDNA is a key gap: one that could benefit from deeper crosstalk with advances in bioconjugation, polymer chemistry, and even natural product-inspired reactivity. Furthermore, enzyme engineering holds untapped potential to expand the range of non-native nucleotides that can be incorporated biosynthetically, reducing the burden on downstream functionalization [14,27,29].

These challenges are formidable but surmountable with collaborative, interdisciplinary approaches. Partnerships across synthetic chemistry, chemical biology, process engineering, polymer physics, and bioinformatics will be needed to push DNA biotechnology beyond its current scale limitations. Equally important is building robust, modular toolkits that academic and industrial researchers can adopt without requiring specialized infrastructure or prohibitive costs. Looking ahead, the roadmap for advancing DNA-based materials must also address practical translation pathways. For industrial adoption, cost competitiveness with existing polymers, predictable performance in complex environments, and compliance with regulatory standards for purity and safety will be decisive.

This review highlights that many promising methods have been demonstrated at the microgram- and milligram-scale but the leap to multi-gram or kilogram production requires systematic evaluation of reaction efficiency, scalability, and sustainability. By re-examining both recent and foundational chemistries through the lens of practical scale-up, researchers can identify the bottlenecks that matter most and develop best practices for each step: sourcing, modification, purification, and characterization. Ultimately, unlocking the next generation of functional DNA materials will depend on how effectively the community closes the gap between design flexibility and manufacturing practicality. As the field matures, answering critical questions (see Outstanding questions) will help determine whether DNA remains a niche nanomaterial or evolves into a robust, scalable polymer platform for advanced biotechnology, bioengineering, and regenerative medicine.

Declaration of interests

No interests are declared.

Resources

[i]www.thermofisher.com/order/catalog/product/SD0011

[ii]https://flacktek.com

[iii]www.avantorsciences.com/pr/en/support/tech-articles/masterflex/shear-sensitive-pumping-with-peristaltic-pumps

[iv]www.michael-smith-engineers.co.uk/resources/useful-info/pumps-for-shear-sensitive-fluids

[v]www.sartorius.com/en/applications/biopharmaceutical-manufacturing/regenerative-medicine/gene-therapy/pdna-production

[vi]www.cytivalifesciences.com/en/us/shop/chromatography/membranes?srsltid=AfmBOorMWIUB3-9gQXbVaWPBOTQ35mMWqZjpll6lR2TqiKiMqqdz_c5_

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