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Redesigning the Genetic Polymers of Life

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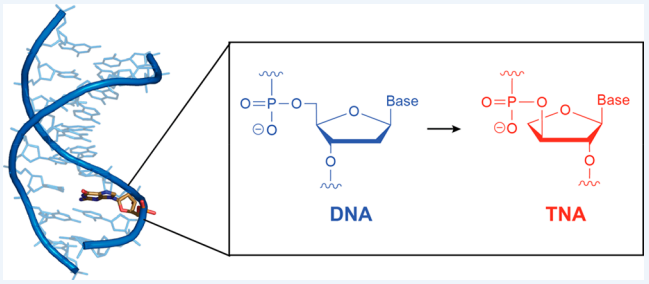
CONSPECTUS: Genomes can be viewed as constantly updated memory systems where information propagated in cells is refined over time by natural selection. This process, commonly known as heredity and evolution, has been the sole domain of DNA since the origin of prokaryotes. Now, some 3.5 billion years later, the pendulum of discovery has swung in a new direction, with carefully trained practitioners enabling the replication and evolution of "xeno-nucleic acids" or "XNAs"—synthetic genetic polymers in which the natural sugar found in DNA and RNA has been replaced with a different type of sugar moiety. XNAs have attracted significant attention as new polymers for synthetic biology, biotechnology, and medicine because of their unique physicochemical properties that may include increased biological stability, enhanced chemical stability, altered helical geometry, or even elevated thermodynamics of Watson−Crick base pairing.



This Account describes our contribution to the field of synthetic biology, where chemical synthesis and polymerase engineering have allowed my lab and others to extend the concepts of heredity and evolution to synthetic genetic polymers with backbone structures that are distinct from those found in nature. I will begin with a discussion of α-L-threofuranosyl nucleic acid (TNA), a specific type of XNA that was chosen as a model system to represent any XNA system. I will then proceed to discuss advances in organic chemistry that were made to enable the synthesis of gram quantities of TNA phosphoramidites and nucleoside triphosphates, the monomers used for solid-phase and polymerase-mediated TNA synthesis, respectively. Next, I will recount our development of droplet-based optical sorting (DrOPS), a single-cell microfluidic technique that was established to evolve XNA polymerases in the laboratory. This section will conclude with structural insights that have been gained by solving X-ray crystal structures of a laboratory-evolved TNA polymerase and a natural DNA polymerase that functions with general reverse transcriptase activity on XNA templates.

The final passage of this Account will examine the role that XNAs have played in synthetic biology by highlighting examples in which engineered polymerases have enabled the evolution of biologically stable affinity reagents (aptamers) and catalysts (XNAzymes) as well as the storage and retrieval of binary information encoded in electronic word and picture file formats. Because these examples provide only a glimpse of what the future may have in store for XNA, I will conclude the Account with my thoughts on how synthetic genetic polymers could help drive new innovations in synthetic biology and molecular medicine.

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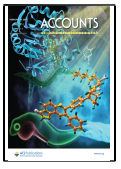

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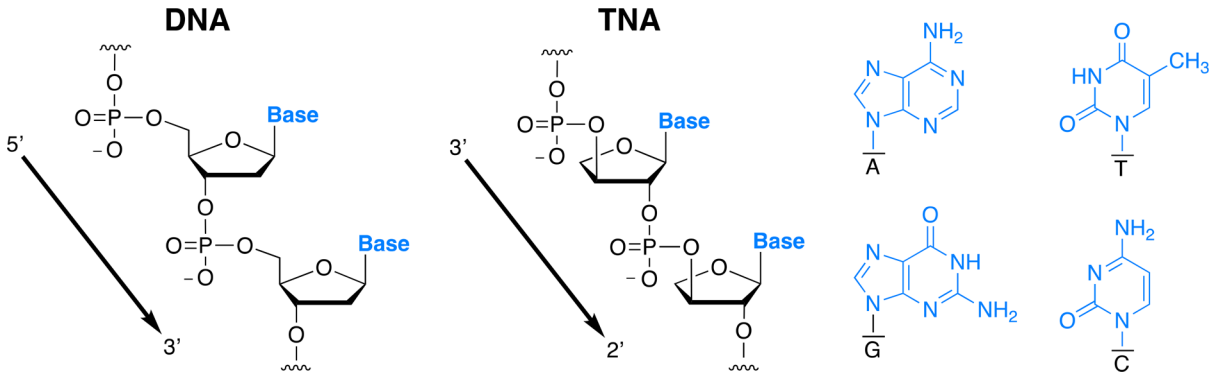

Figure 1. Chemical structures of DNA and TNA. Constitutional structures for the linearized backbones of DNA and α-L-threofuranosyl-(3′,2′)-nucleic acid (TNA) are shown. TNA has one less atom per backbone repeat unit than natural DNA and maintains a strand polarity of 3′ → 2′ rather than the more normal strand polarity of 5′ → 3′ found in natural DNA and RNA.

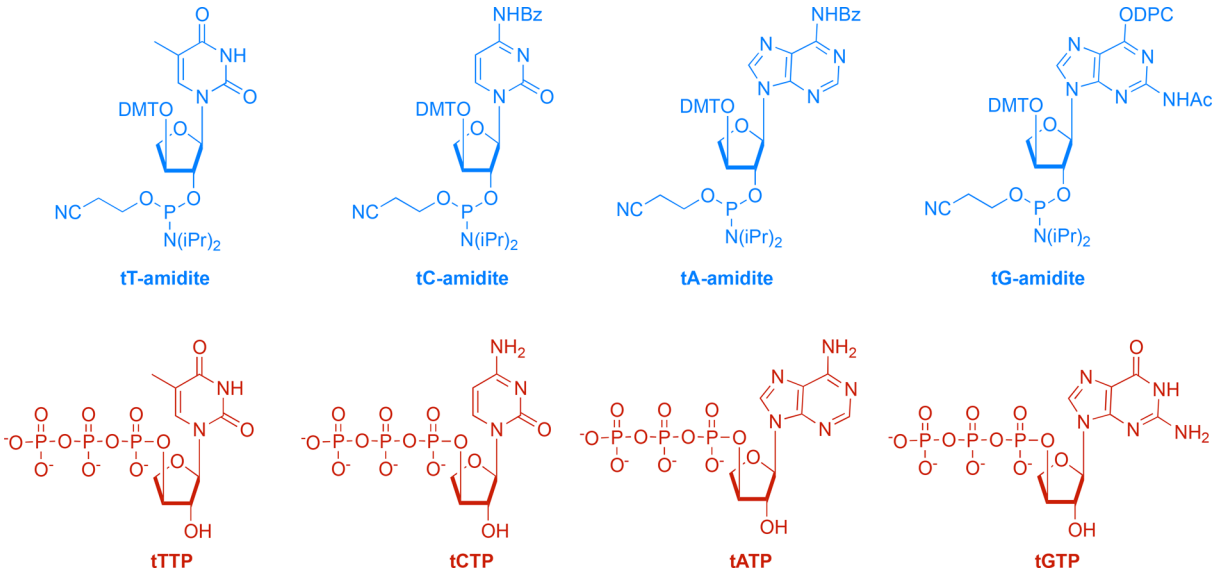

Figure 2. Chemical structures of TNA phosphoramidites and nucleoside triphosphates. TNA phosphoramidites (blue) and nucleoside triphosphates (red) are building blocks for solid-phase and polymerase-mediated TNA oligonucleotide synthesis.

stability. *J. Am. Chem. Soc.* **2020**, *142*, 7721−7724.[4] *This synthetic biology paper describes a new approach for evovling XNA aptamers in the laboratory by linking XNAs to their encoding DNA templates.*

1. INTRODUCTION

1.1. What is XNA?

The term "xeno-nucleic acid", abbreviated "XNA", first appeared in the literature in 2009 in a theoretical paper describing the potential for synthetic genetic polymers (XNAs) to increase the biosafety of genetically modified organisms by storing synthetic biology information in a genetic material that is orthogonal to the host genome.[5] As described by Herdewijn and Marlière, XNAs were defined as nucleic acid molecules whose backbone structures are constructed from sugars that are distinct from those found in DNA and RNA.[5] This definition makes it easy to distinguish XNAs from other types of chemical modifications, such as those found on the nucleobases of DNA or RNA nucleotides.[6] While it is unlikely that we will see XNA-modified organisms anytime soon,[7] the basic idea of encoding information in synthetic genetic polymers helped launch a new area of synthetic biology called *synthetic genetics* in which researchers are working to develop a molecular biology toolkit of enzymes (e.g., polymerases, kinases, ligases, etc.) that act on XNA.[8] Toward this goal, the discovery of engineered polymerases that can faithfully copy genetic information back and forth between DNA and XNA was considered a critical first step in the process, as such enzymes would make it possible to synthesize, propagate, and evolve XNA molecules in a test tube.[9]

1.2. A Model System Based on TNA

Our foray into the field of synthetic genetics began long before the term synthetic genetics was coined.[10] Inspired by Eschenmoser's chemical evaluation of artificial genetic polymers constructed from different aldose sugars,[11] we wished to establish a Darwinian evolution system that would make it possible to explore the functional properties of different XNA systems by in vitro selection. We focused our studies on α-L-threofuranosyl nucleic acid (TNA) (Figure 1),[12−14] a synthetic genetic material composed of repeating α-L-threofuranosyl nucleotides connected by quasi-trans-diaxial 2′,3′-phosphodiester linkages.[15] TNA is one of the few synthetic genetic systems that is able to form stable antiparallel Watson−Crick duplex structures with complementary strands of DNA and RNA.[15−17] This was a surprising result at the time, as the

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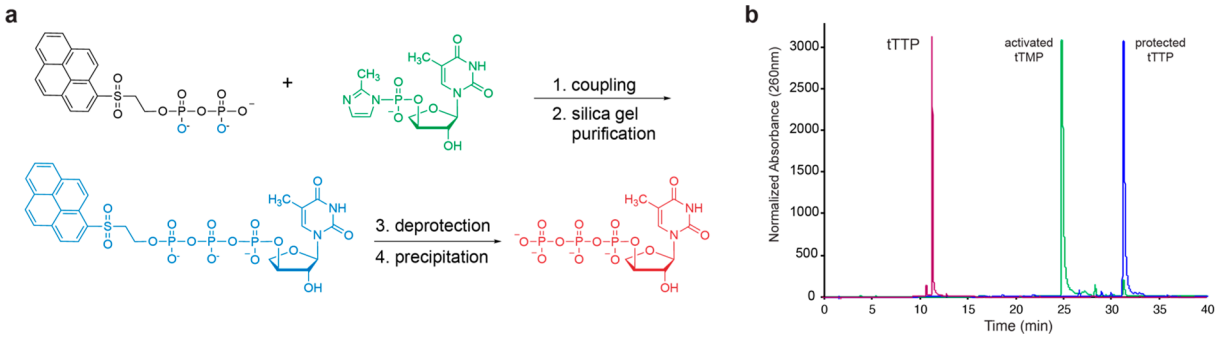

Figure 3. TNA triphosphate synthesis. (a) General scheme for tNTP synthesis from activated 3′-nucleoside monophosphate and pyrene pyrophosphate. (b) HPLC analysis of key intermediates shown in (a). Color scheme: tTTP (red), activated tTMP (green), and protected tTTP (blue). Adapted from ref 44. Copyright 2019 American Chemical Society.

backbone repeat unit of TNA is one atom shorter than that of DNA and RNA.[18] Crystal structures solved by Egli and coworkers helped to clarify this paradox by providing a cross-pairing model in which the rigid TNA backbone forces the complementary DNA or RNA strand to adopt a sugar conformation that reduces the P−P bond distances along natural strand.[19] Other observations, such as why TNA cross-pairs more strongly with RNA than DNA, are less well understood but could be due to the fact that TNA, like RNA, favors an A-form helical geometry.[20]

Our decision to pursue TNA as a model system for synthetic genetics was influenced by a number of important factors. First, TNA is recalcitrant to nucleases that degrade DNA and RNA, making it an ideal material for future diagnostic and therapeutic applications that require high biological stability.[21] Second, the ability of TNA to transfer genetic information to DNA via complementary base pairing enables practitioners to take advantage of new technologies that are continually being developed for high-throughput DNA synthesis and sequencing.[22,23] Third, TNA was a new genetic system with unknown structural and functional properties, providing an avenue for future growth in a new area of science. Fourth, TNA required chemical synthesis, which limited competition by other laboratories. The last two were strategic considerations intended to allow a new laboratory to avoid the perils of working in a crowded field, something that I would recommend all young scholars to consider when starting their careers.

2. CHEMICAL SYNTHESIS

From DNA synthesis to RNA therapeutics, organic chemists have laid the foundation for nucleic acid technologies that have revolutionized synthetic biology and healthcare. Synthetic genetics is no exception, as some of the most interesting XNAs are not yet commercially available and must be prepared by students trained in the art of chemical synthesis. Although a detailed review of XNA synthesis is beyond the scope of this Account, I would like to direct readers interested in this subject to an excellent compendium on the chemistry of artificial nucleic acid polymers and a comprehensive review of nucleic acid chemistry.[24,25] The following section highlights the chemistry that my laboratory established to enable the production of large quantities of TNA phosphoramidite and nucleoside triphosphate monomers (Figure 2), the building blocks required for solid-phase and polymerase-mediated TNA synthesis, respectively.

2.1. TNA Phosphoramidites

Following our description of the first TNA aptamer in 2012,[26] we quickly realized that TNA synthesis had become a bottleneck in our research program. The synthetic strategy established by Eschenmoser and colleagues, though novel, suffered from a number of shortcomings that precluded the large-scale synthesis of TNA monomers. This included poor overall yield (∼2−6%), expensive reagents, extensive silica gel purification, and limited regioselectivity of the 2′ and 3′ hydroxyl groups.[15] Seeking to overcome these challenges, we redesigned the synthetic route from vitamin C to TNA nucleosides.[27] The new strategy solved the regioselectivity problem using an orthogonal protecting group strategy that unambiguously distinguishes the 2′ and 3′ hydroxyl groups of the threose sugar.[28] Other important changes included the use of oxalic acid as an inexpensive calcium exchange reagent in place of the more expensive Dowex cation exchange resin and the elimination of all silica gel purification steps for early intermediates. Together, these changes made it possible to produce ∼25 g of nucleoside from 100 g of vitamin C in 10 synthetic steps in an overall yield of 16−23% depending on the nucleoside.[15] Our synthetic approach has since been adopted by other laboratories interested in evaluating the properties of TNA oligonucleotides[29−31] and was used as the basis for constructing several sugar- and base-modified TNA analogues in my laboratory.[32−37]

2.2. TNA Triphosphates

Natural and chemically modified nucleoside triphosphates have become indispensable reagents for a broad range of biotechnology applications.[38] DNA triphosphates (dNTPs) are commonly manufactured using enzymatic protocols, while modified triphosphates are produced by chemical synthesis.[39,40] In general, enzymatic strategies suffer from high substrate specificity, while synthetic routes face reagent compatibility problems, difficult reaction conditions, and low yields. Once synthesized, nucleoside triphosphates need to be purified by high-performance liquid chromatography (HPLC), which is a tedious process that limits the scale of purified compound to tens of milligrams per week in a typical academic environment.

Recognizing the need for hundreds of milligrams of TNA triphosphates (tNTPs), we began to explore new approaches for constructing tNTPs.[37,41−43] This work eventually led us to invent a new P(V) phosphorylating reagent called pyrene pyrophosphate that enables the synthesis of nucleoside triphosphates on scales vastly exceeding those achievable by traditional routes that necessitate HPLC (Figure 3).[44] In this approach, activated nucleoside monophosphates synthesized in

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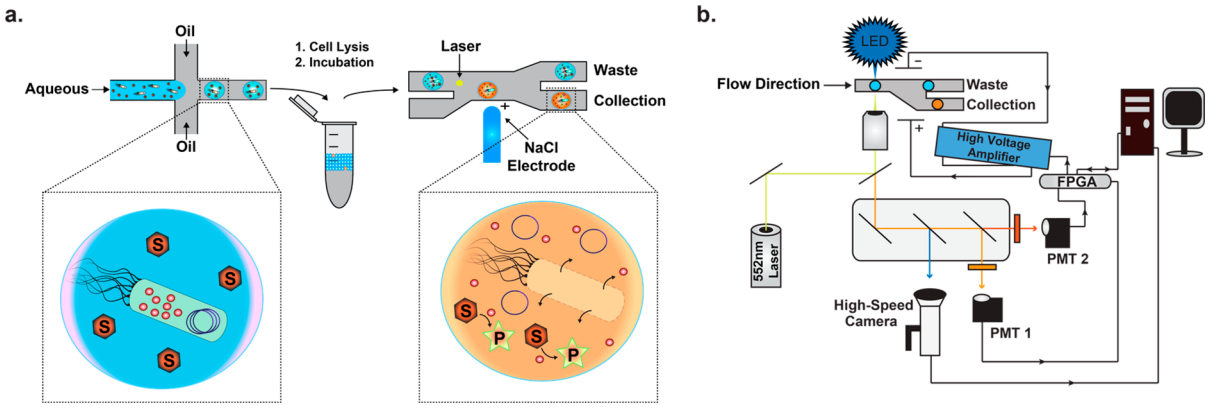

Figure 4. Droplet-based optical polymerase sorting (DrOPS). (a) Overview of droplet production and sorting. (b) Overview of the optical train and electronic components of the FADS system. Reproduced from ref 2. Copyright 2019 American Chemical Society.

protected form are coupled to the pyrene pyrophosphate reagent to produce nucleoside triphosphate derivatives that are purified by silica gel chromatography on scales ranging from 50 to 500 mg. The desired compounds are then generated as sodium salts following deprotection and precipitation. This strategy, which is efficient, inexpensive, and operationally straightforward, was successfully demonstrated on a diverse set of natural and modified nucleoside triphosphates, including natural dNTPs, mirror-image L-dNTPs, tNTPs, and 3′-phosphorylated dNTPs.[44] The pyrene pyrophosphate strategy is currently the method of choice for synthesizing tNTPs in the laboratory.

3. TNA REPLICATION

Synthetic genetics requires engineered polymerases that can synthesize, propagate, and evolve genetic information encoded in synthetic genetic polymers.[8] A typical replication cycle involves reverse transcription of XNA into DNA, amplification of the DNA using the polymerase chain reaction (PCR), separation of the strands, and forward transcription of the DNA back into XNA.[45] Because amplification occurs at the DNA level, existing XNAs are not yet capable of true replication.[46] For this to happen, XNAs would need to amplify their own genetic information by copying one type of XNA directly into the same XNA. Nevertheless, significant progress has been made in evaluating the properties of XNA polymers using replication systems that copy genetic information back and forth between DNA and XNA.[47] The following section describes our approach to establishing enzymes that can replicate TNA in vitro.

3.1. Evolution of a DNA-Dependent TNA Polymerase

Our first attempt at discovering polymerases that could recognize TNA substrates, either in the template or as nucleoside triphosphates, involved screening known polymerases for activity in a standard primer extension assay.[12−14] Those low-throughput assays were intended to identify candidate polymerases that could be improved by directed evolution. Our screening effort led to the observation that hyperthermophilic archaeal B-family DNA polymerases were more tolerant of TNA nucleotides than other classes of polymerases.[14] At the time, the most active TNA polymerase identified was Therminator DNA polymerase,[48] an engineered variant of 9°N DNA polymerase developed by New England Biolabs for Sanger sequencing.[49] Therminator carries a bulky A485L mutation in the finger subdomain that is thought to change the dynamics between the open and closed conformations of the polymerase when sugar-modified nucleotides are present in the enzyme active site.[50] This mutation, as well as other bulky mutations at this position, occur in all of the engineered polymerases that have been developed to synthesize XNA.[9] Unfortunately, Therminator required mutagenic manganese ions for activity and stalled when copying G-rich sequences into TNA, making it impossible to synthesize unbiased libraries of TNA oligonucleotides.[26]

To overcome the limitations of Therminator DNA polymerase, my laboratory established a general strategy for evolving new polymerase functions called droplet-based optical polymerase sorting (DrOPS).[1] DrOPS is a high-throughput single-cell approach that combines the ultrafast screening power of microfluidics with the high sensitivity of optical sorting. With this technique (Figure 4), a polymerase library is expressed in *Escherichia coli*, and single cells are encapsulated in uniform droplets containing a fluorescent sensor that is responsive to polymerase activity. The surrounding oil acts as a barrier that prevents the contents of one droplet from mixing with the contents of another droplet. The latest microfluidic designs are capable of generating ∼20 μm droplets at a rate of 30 kHz, allowing for the production of >10^8 droplets/h.[2] Following droplet production, each polymerase variant and its encoding plasmid are released into the droplet by lysing the *E. coli* with heat. Polymerases that successfully copy the template into full-length product produce a fluorescent signal by triggering an optical sensor. The droplet population is then sorted using a custom microfluidic fluorescence-activated droplet sorting (FADS) device that was established to facilitate the directed evolution of XNA-modifying protein enzymes (e.g., polymerases, kinases, ligases, etc.).[2]

The DrOPS technique has several advantages over existing polymerase evolution technologies.[9] First, DrOPS provides an economical approach to polymerase engineering by allowing researchers to use ∼10^6-fold less sample than is typical for polymerase screening.[51] This aspect of the technology is particularly attractive for XNA projects that require chemically synthesized substrates. Second, DrOPS uses a microfluidic technology that allows researchers to screen ∼10^8 variants per day, vastly exceeding all known robotic screening protocols. Third, the technology provides enormous control over the composition of the primer, template, and nucleoside triphosphates, making it possible to select for any type of polymerase function (i.e., transcription, reverse transcription, replication). Fourth, it relies on physical methods for sorting individual droplets, which increases the partitioning efficiency while

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reducing the occurrence of background DNA contamination associated with bead-based enrichment protocols.

DrOPS has been used successfully in a number of polymerase evolution studies.[1,52,53] Most recently, DrOPS was combined with programmed allelic mutagenesis to exhaustively evaluate all possible single-point mutations in the entire catalytic domain (~400 amino acids) of a naturally occurring replicative DNA polymerase isolated from the hyperthermophilic archaeal species *Thermococcus kodakarensis* (Kod). This effort led to the discovery of Kod-RSGA, which is the best TNA polymerase developed to date and carries the RSGA mutations along with two exonuclease-silencing mutations. Kod-RSGA functions at a catalytic rate of ~10 nt/min with an overall error rate of 9.5×10^{-3} (99.1% fidelity).[53] The RSGA mutations are located at disparate positions within the finger and thumb subdomains, which are responsible for recognizing the incoming tNTP substrate and primer−template duplex, respectively. Presumably, additional gains in TNA synthesis activity could be achieved by identifying new mutations in the palm subdomain, which contains the active-site residues responsible for phosphodiester bond formation.

3.2. A Natural Polymerase with General XNA Reverse Transcriptase Activity

The process of reverse-transcribing XNA back into DNA for amplification by PCR was originally thought to be a challenging problem, as the first polymerase engineering technologies were all based on DNA-templated strategies.[54] Since this work was done prior to our DrOPS technology, we chose to evaluate a previous low-throughput screen of commercial polymerases for hits that could be optimized by adjusting the enzyme concentration, temperature, pH, or buffer conditions. In that study, a naturally occurring DNA polymerase I variant isolated from the thermophilic species *Geobacillus stearothermophilus* (Bst) was found to be capable of full-length DNA synthesis on a short TNA template.[13] Through careful optimization of the reaction conditions, we were able to show that Bst was able to copy longer TNA templates (~70 nucleotides) into DNA.[55] In subsequent work, we have extended this finding to other XNA templates, which now include 2′-fluoroarabino nucleic acid (FANA), arabino nucleic acid (ANA) (unpublished), and hexitol nucleic acid (HNA) (unpublished). These results indicate that Bst DNA polymerase functions with general XNA reverse transcriptase activity, making it the only known natural polymerase to recognize XNA templates with diverse chemical compositions.

3.3. Enzymatic Ligation of TNA Oligonucleotides

Enzymatic ligation has been proposed as a general strategy for assembling XNA strands of lengths beyond what is currently possible by solid-phase synthesis.[56] Toward this goal, we evaluated the substrate specificity of several naturally occurring DNA and RNA ligases for the ability to recognize TNA oligonucleotides in a DNA-templated nick joining assay.[57] Systematic screening and subsequent reaction condition optimization identified the bacteriophages T7, T4, and T3 DNA ligase as enzymes capable of synthesizing TNA−DNA, DNA−TNA, and TNA−TNA oligonucleotide products by template-directed ligation. Analysis of the 16 possible dinucleotide combinations at the ligation junction revealed that T3-mediated TNA−TNA ligation exhibited a strong sequence-dependent bias with a clear preference for A−T and A−G dinucleotide junctions. We suggest that these results provide a starting point for identifying TNA ligase variants with improved activity for TNA substrates by directed evolution.

4. TNA POLYMERASE STRUCTURES

Engineered polymerases established to support the replication of XNA polymers in the laboratory all derive from hyperthermophilic archaeal B-family DNA polymerases.[9] This includes XNA reverse transcriptases that have recently been evolved to recognize different XNA templates.[58] Hyperthermophilic archaeal B-family DNA polymerases represent a highly conserved family of enzymes that adopt similarly folded structures such that the mutations discovered in one family member recapitulate when inserted into the backbone structure of another family member.[59] However, despite their importance in a range of biotechnology applications,[38] no structural information was available for the ternary complex of an archaeal B-family DNA polymerase prior to our work in this area. This changed in 2017, when my lab and the laboratory of Andreas Marx published contemporaneous papers on the ternary structures of engineered and natural archaeal B-family DNA polymerases, respectively.[3,60] More recently, the apo and binary complexes for the HNA polymerase Tgo-6G12 were reported.[61] The following section describes insights into the mechanism of TNA replication that have been gained using crystal structures that were solved for two model polymerases that facilitate the synthesis and reverse transcription of TNA sequences.

4.1. Structural Basis of TNA Synthesis

In an effort to better understand the structural basis for TNA synthesis by an engineered TNA polymerase, we solved five X-ray crystal structures that capture the pathway by which tNTPs are selected and extended in a template-dependent manner using a first-generation laboratory-evolved TNA polymerase known as Kod-RI.[3] The structures, which include the apo, binary, open, and closed ternary structures and the translocated product, detail an ensemble of interactions and conformational changes that are required to promote TNA synthesis. Of particular importance were the open and closed ternary structures of Kod-RI, which reveal a suboptimal geometry for the incoming tNTP that is characterized by severe buckle and propeller distortions to the nascent base pair. This suboptimal binding geometry, which extends to the bound divalent metal ions, likely accounts for the low rate of catalysis (~1 nt/min) observed for Kod-RI-mediated TNA synthesis (Figure 5).[62] By comparison, the ternary structure of wild-type Kod DNA polymerase solved by Marx and co-workers exhibits a coplanar geometry for the nascent base pair with the divalent metal ions ideally positioned for chemical bond formation (Figure 5).[60] More recently, a crystal structure of Kod-RSGA, a third-generation TNA polymerase that is 10-fold faster than Kod-RI, yielded a tNTP substrate in the enzyme active site with improved base-pairing geometry (unpublished data). This latter finding suggests that the remodeling of the active site by directed evolution allowed Kod-RSGA to better accommodate tNTP substrates.

4.2. Structural Basis of TNA Reverse Transcription

Replicative DNA polymerases have evolved over millions to billions of years to recognize DNA substrates with high specificity and high fidelity. For example, *E. coli* DNA polymerase I is able to discriminate dNTPs from NTPs by a factor of up to 10^5-fold even though the molecular difference between dNTP and NTP is the presence of a 2′-hydroxyl group on the ribose sugar.[63] One interesting exception to this paradigm

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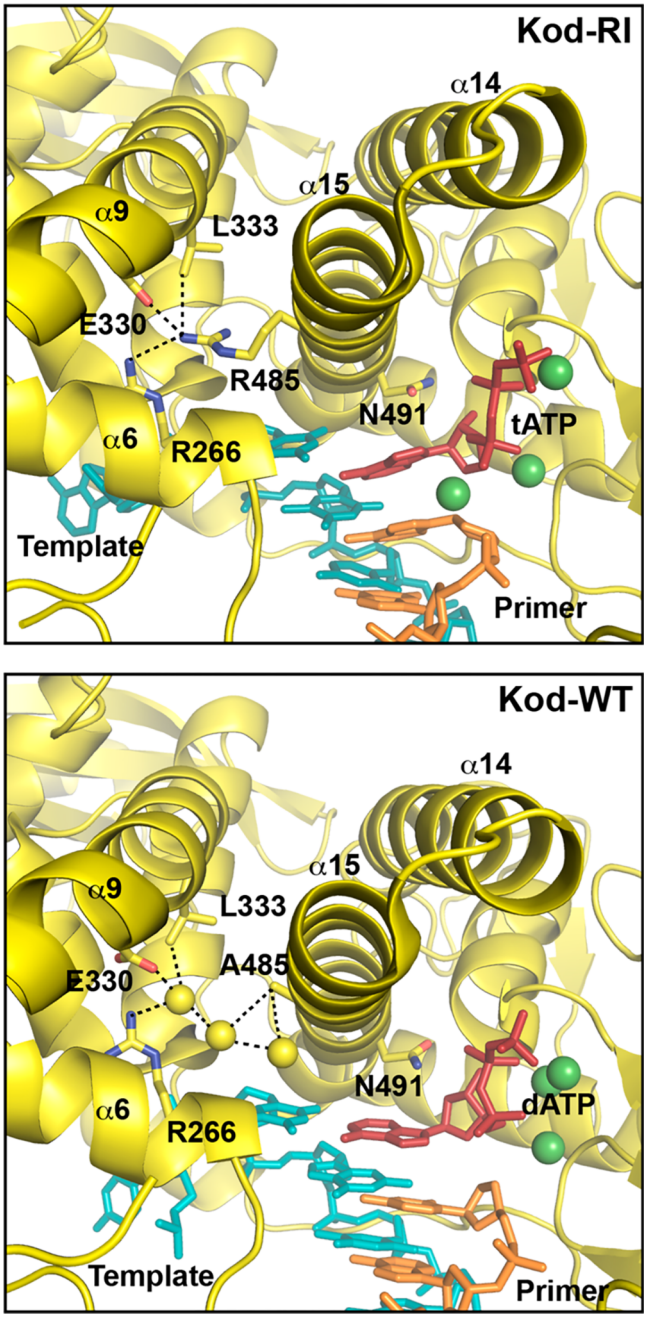

Figure 5. Crystal structures of (top) the closed ternary complex of Kod-RI (PDB entry 5VU8) and (bottom) wild-type Kod (PDB entry 5OMF). The active-site region depicts the geometry of the incoming nucleotide. Color scheme: protein (yellow), template (blue), primer (orange), incoming triphosphate (red), and magnesium ions (green). Reproduced from ref 52. Copyright 2019 American Chemical Society.

is the naturally occurring bacterial DNA polymerase I member Bst DNA polymerase discussed above, which exhibits an innate ability to reverse-transcribe XNA templates of diverse chemical composition.[55,64] In 2019 we published two high-resolution X-ray crystal structures of Bst DNA polymerase (Figure 6) that capture the post-translocated product of DNA synthesis on templates composed entirely of FANA and TNA, respectively.[65] Analysis of the active-site region in both crystal structures indicates that the O and O1 helices of the finger subdomain and a loop connecting the β12 and β13 strands of the palm subdomain adjust to compensate for differences in the helical

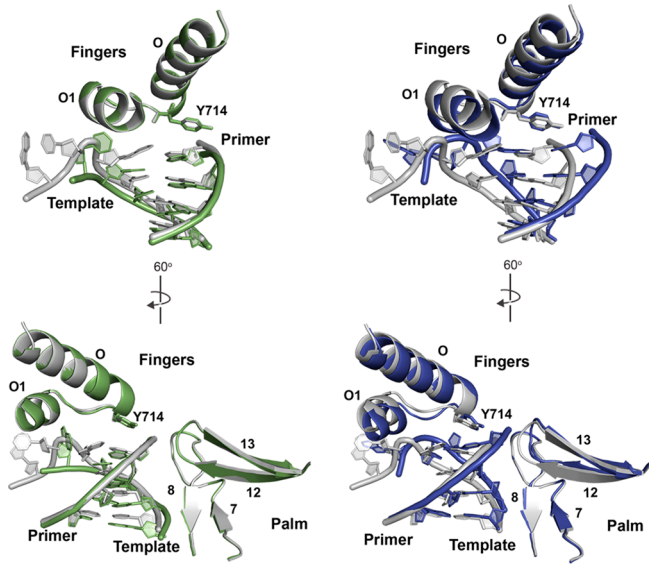

Figure 6. Crystal structures of Bst DNA polymerase bound to (left) FANA (green, PDB entry 6MU4) and (right) TNA (blue, PDB entry 6MU5) templates. Both structures are overlaid with the natural system (gray, PDB entry 6DSY). From ref 65. CC BY 4.0.

geometry of the chimeric heteroduplex.[65] These observations suggest that structural plasticity may play a role in the mechanism of XNA-dependent DNA synthesis by Bst DNA polymerase. Further studies are now needed to better understand why a natural DNA polymerase is able to recognize different XNA templates with reasonable efficiency and high fidelity.

5. APPLICATIONS

One of the more exciting areas of synthetic genetics involves applying XNA reagents toward the development of molecular tools that address societal problems.[66,67] Although this field is still very much in its infancy, my laboratory and others have already established examples of XNAs that function with specific ligand binding affinity or catalytic activity.[4,10,26,31,35,64,68−70] Affinity reagents produced in these studies represent the next generation of nucleic acid sensors, diagnostics, and therapeutics.[71] XNA enzymes, especially those that cleave target RNA messages, could one day find practical utility in gene-silencing assays that are designed to characterize and treat genetic diseases caused by single-nucleotide polymorphisms.[72] The capacity for XNAs to store genetic information in linear polymers that are resistant to nuclease digestion offers a new area of research where XNAs function as novel biomaterials for data archiving.[73] The following section highlights a few examples where XNA reagents are being developed for new or improved applications in synthetic biology, biotechnology, and healthcare.

5.1. Affinity Reagents

Aptamers, which are nucleic acid molecules that mimic antibodies by folding into shapes with specific target binding affinity, have enormous potential as molecular recognition agents in sensors, point-of-care diagnostics, and next-generation therapeutics.[71] TNA is an attractive system for these applications because TNA is recalcitrant to nuclease digestion, making it an ideal reagent for biological conditions where DNA and RNA rapidly degrade.[21] Our initial experiments into the evolution of TNA aptamers were largely proof-of-principle demonstrations designed to show that the principles of heredity

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and evolution could be extended to synthetic genetic polymers with non-natural sugar–phosphate backbone structures.[26,35,55] Once confident that we could produce TNA aptamers by in vitro selection, our goal shifted toward new technologies that would allow high-affinity TNA aptamers to be isolated from minimal rounds of in vitro selection.

As a first step in this new direction, we established an in vitro selection system that simplified the selection protocol by bypassing the need for an XNA reverse transcriptase enzyme (Figure 7).[74] The approach, termed DNA display, is conceptually similar to the protein evolution technique of mRNA display, whereby a genotype–phenotype relationship is created by covalently linking freshly translated proteins to their encoding mRNA strands.[75] In the case of DNA display, the genotype–phenotype link is maintained using a self-priming DNA template that allows newly synthesized TNA molecules to be covalently attached to their encoding DNA template, which is made double-stranded (ds) through a second primer-extension step performed on the DNA template. The DNA display strategy allows for stringent washing to be employed during the selection protocol, as the dsDNA region of DNA–TNA aptamers that remain bound to the target can be directly amplified by PCR. In a proof-of-principle demonstration, we successfully evolved a population of biologically stable TNA aptamers that bound to human HIV reverse transcriptase with K_D values in the range of 0.4–4 nM.[4] On the basis of these results, we are confident that further advances in XNA technology could narrow the gap between aptamers and monoclonal antibodies in practical applications involving analyte detection.

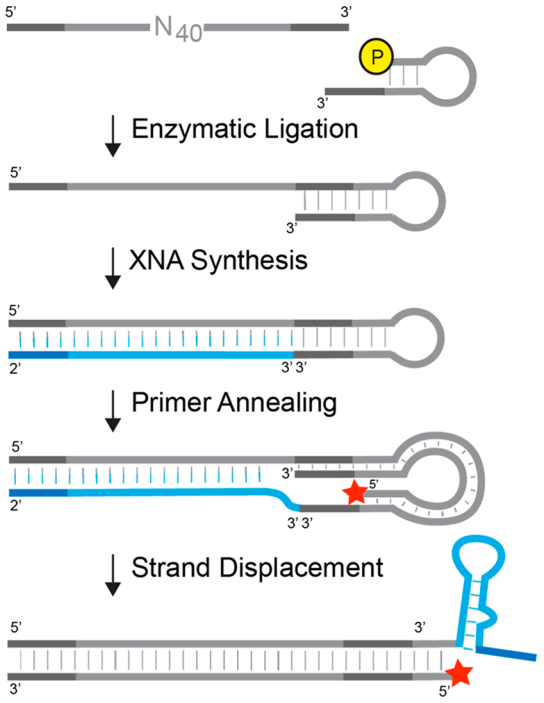

Figure 7. DNA display. TNA molecules are displayed on their encoding DNA template by extending a self-priming template with TNA. The DNA template is made double-stranded through a second primer extension with DNA. Reproduced from ref 4. Copyright 2020 American Chemical Society.

5.2. Catalysts

In recent years, XNA technologies have enabled the isolation of XNA enzymes with trans-acting endonuclease and ligase activities. The first examples were reported by Holliger and colleagues on catalysts derived from XNAs with four different backbone chemistries (FANA, ANA, HNA, and cyclohexenyl nucleic acid (CeNA)).[70] These results unambiguously showed that XNAs, like DNA and RNA before them,[76,77] have the capacity to catalyze chemical reactions on oligonucleotide substrates. However, the XNAzymes produced in these selections functioned with poor catalytic activity, raising the question of whether XNAs can achieve classic multiple turnover kinetics commonly observed in protein enzymes. In 2018, my laboratory addressed this question by evolving an RNA-cleaving FANAzyme that exhibits classic Michaelis–Menten kinetics with multiple turnover activity.[64] This study also showed that FANAzymes can be evolved using purely commercial reagents, opening the field of synthetic genetics to a larger scientific community.[64] Interestingly, subsequent studies revealed that our FANAzyme recognizes DNA substrates with a central *ribo*G residue more efficiently than DNAzymes that had been intentionally developed to cleave the identical substrate.[78] Further studies are now underway to elucidate the molecular basis of this enhanced catalytic activity.

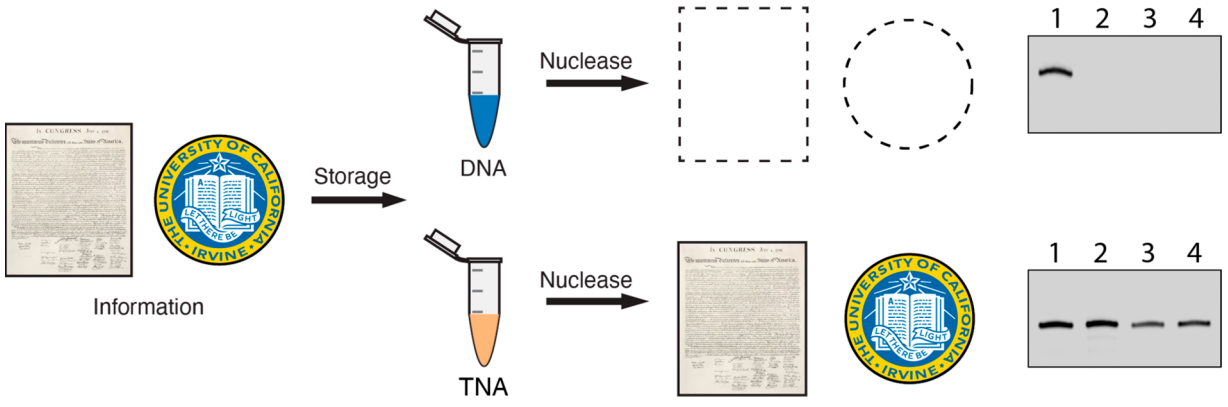

Figure 8. Information storage. TNA storage is accomplished by converting digital files into DNA and then copying the DNA into TNA. File recovery involves copying the TNA back into DNA, sequencing the DNA, and reassembling the binary information. Information stored in TNA is biologically secure, while equivalent information stored in DNA degrades when nucleases are present. Reproduced from ref 73. Copyright 2020 American Chemical Society.

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5.3. Information Storage

In addition to healthcare applications, XNAs are gaining ground on fundamental problems that impact data storage. In recent years, it has become apparent that traditional data storage systems are struggling to keep pace with the growing volume of information generated on an annual basis. DNA has been proposed as a possible solution to this problem, as it provides a well-understood soft material for low-energy, high-density information storage.[79] At a theoretical maximum, 1 g of DNA could store 455 exabytes (4.55×10^{20} bytes) of information, equivalent to one-quarter of the world's annual data production.[80] One significant drawback of using DNA as a biopolymer for information storage is its susceptibility to nucleases present in the environment. Fortunately, this problem can be easily overcome using XNAs, like TNA, that are completely resistant to nuclease digestion. In a proof-of-concept demonstration (Figure 8), we used a simple genetic coding strategy to convert two digital files—a word file containing the Declaration of Independence and a picture file of the UCI seal—into TNA.[73] The data (23 kilobytes) were converted into a bit stream that was encoded in 7451 TNA oligonucleotides and recovered with perfect accuracy, making it the largest XNA fidelity study performed at the time that this Account was written. When exposed to biological nucleases, the TNA message remained intact, while the equivalent DNA message was completely destroyed. We suggest that this approach to data archiving provides a safeguard against unexpected information loss caused by nuclease exposure. Further improvements in TNA polymerase technology, especially with the future generation of a TNA polymerase capable of PCR amplification, would greatly enhance the capacity for information storage in synthetic TNA polymers.

6. SUMMARY AND OUTLOOK

The concepts of heredity and evolution form the cornerstone of biology by providing a mechanism for information to pass from one generation to the next through genome replication and cell division. In the laboratory, we and others have shown that these concepts can also be extended to synthetic genetic polymers with backbone architectures that are distinct from those found in nature. Although not a direct goal of my research, the possibility of similar genetic materials replicating in organisms that exist in worlds beyond our own is intriguing and represents the potential of synthetic genetic systems to impact other fields of science. Investigating the molecular basis of information storage in these and other XNA systems may aid in the design of experiments that search for signatures of life elsewhere in the universe and lead to a better understanding of basic principles of nucleic acid structure and function.

Looking ahead to the future, it is tempting to speculate how XNAs could impact humanity in a meaningful way. Although it is always difficult to predict the future, areas where XNAs could have a significant impact include (1) gene silencing, especially in the design of new reagents that can distinguish allele-specific mutations associated with protein targets that are currently considered undruggable; (2) new point-of-care diagnostic agents for personalized medicine that allow physicians to rapidly diagnose patients presenting with respiratory illnesses, including COVID-19; (3) new substitutes for therapeutic antibodies that are easier to synthesize and distribute to patients; (4) and orthogonal approaches to genetically engineered organisms with built-in controls that safeguard against environmental escape.

For these goals to be realized, further improvements are needed in the methodology used to generate XNA building blocks and the enzymes that recognize them. Fortunately, research into the synthesis and replication of synthetic genetic polymers is steadily growing, providing reasonable assurance that XNA technologies will find increasing use in standard molecular biology laboratories across the globe. We in the Chaput laboratory look forward to the continued transition of synthetic genetics from a specialty science to a general platform that is as widely available as nature's choice, DNA and RNA.

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Notes

The author declares the following competing financial interest(s): The author is a named inventor on patents related to TNA synthesis and replication in vitro.

Biography

John C. Chaput was born and raised in St. Paul, Minnesota. He received his B.S. in Chemistry from Creighton University and a Ph.D. in Chemistry from the University of California, Riverside, with Chris Switzer and completed his postdoctoral training at Harvard Medical School with Jack Szostak. He started his academic career as a founding faculty member of the Biodesign Institute at Arizona State University in 2005 and joined the faculty at the University of California, Irvine, in 2015, where he is currently a professor in the Departments of Pharmaceutical Sciences, Chemistry, and Molecular Biology and Biochemistry. His research interests focus on the development of synthetic genetic polymers for diagnostic and therapeutic applications in molecular medicine.

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