Randomized Fast Design of Short DNA Words*

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Abstract. We consider the problem of efficiently designing sets (codes) of equal-length DNA strings (words) that satisfy certain combinatorial constraints. This problem has numerous motivations including DNA computing and DNA self-assembly. Previous work has extended results from coding theory to obtain bounds on code size for new biologically motivated constraints and has applied heuristic local search and genetic algorithm techniques for code design. This paper proposes a natural optimization formulation of the DNA code design problem in which the goal is to design n strings that satisfy a given set of constraints while minimizing the length of the strings. For multiple sets of constraints, we provide high-probability algorithms that run in time polynomial in n and any given constraint parameters, and output strings of length within a constant factor of the optimal. To the best of our knowledge, this work is the first to consider this type of optimization problem in the context of DNA code design.

1 Introduction

In this paper we study the problem of efficiently designing sets (codes) of DNA strings (words) of near optimal length that fulfill certain combinatorial constraints. Many applications have emerged in recent years that depend on the scalable design of such words. One such problem is in DNA computing where inputs to computational problems are encoded into DNA strands for the purpose of computing via DNA complementary binding [1]. Another application involves implementing Wang tile self-assembly systems by encoding glues of Wang tiles into strands of DNA [17]. DNA words can also be used to store information at the molecular level [4], act as molecular bar codes for identifying molecules in complex libraries [4, 5, 13], or implement DNA arrays [3].

For a set of DNA words to be effective for the above applications, they must fulfill a number of combinatorial constraints. Of particular importance is the need for specific hybridization between a given word and its unique Watson-Crick complement. That is, we need to make sure that hybridization does not

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Word Length and Time Complexity for DNA Word Design				
	Word Length		Time Complexity	
	Lower Bound Upper Bound		Lower Bound	Upper Bound
$\mathrm{DWD}_{1,2,3,4,5,6,7}$	$\Theta(\ell)$ (Thm. 1)　(Thm. 3)		$\Theta(n\ell)$ (Thm. 1)　　(Thm. 3)	
$\mathrm{DWD}_{1,2,3,7,8}$	$\Theta(\ell)$ (Thm. 1)　(Thm. 4)		$\Theta(n\ell)$ (Thm. 1)　　(Thm. 4)	
$\mathrm{DWD}_{1,2,3,4,5,6,9}$	$\Theta(\ell)$ (Thm. 1)　(Thm. 6)		$\Omega(n\ell)$ (Thm. 1)	$O\left(\min\left\{\begin{array}{l}\ell^{1.5}\log^{0.5}\ell + n\ell, \\ n\ell\log\ell\end{array}\right\}\right)$ (Thm. 6)

Table 1. This table summarizes our results regarding the efficient design of DNA words. Here n is the number of words; k denotes the maximum of the constraint parameters for constraints 1 through 6 (see Section 2); and $\ell = \Theta(k + \log n)$ denotes the optimal achievable word length for the listed word design problems (see Theorems 1, 3, 4 and 6).

occur among a word and the complement of a different word in the set, or even of any word with any other word in the set. For this requirement Marathe et al. [12] have proposed the *basic Hamming constraint*, *reverse complement Hamming constraint*, and *self-complementary constraint*. We further consider the more restricting *shifting Hamming constraint* which requires a large Hamming distance between all alignments of any pair of words [6].

We also consider three constraints not related to Hamming distance. The *consecutive base constraint* limits the length of any run of identical bases in any given word. Long runs of identical bases are considered to cause hybridization errors [6, 14]. The *GC content constraint* requires that a large percentage of the bases in any given word are either G or C. This constraint is meant to give each string similar thermodynamic properties [14–16]. The *free energy constraint* requires that the difference in free energy of any two words is bounded by a small constant. This helps ensure that each word in the set has a similar melting temperature [6, 12].

In addition to the above constraints, it is desirable for the length ℓ of each word to be as small as possible. The motivation for minimizing ℓ is evident from the fact that it is more difficult to synthesize longer strands. Similarly, longer DNA strands require more DNA to be used for the respective application.

There has been much previous work in the design of DNA words [4, 6, 9–13, 15, 16]. In particular, Marathe et al. [12] have extended results from coding theory to obtain bounds on code size for various biologically motivated constraints. However, most work in this area has been based on heuristics, genetic algorithms, and stochastic local searches that do not provide provably good words provably fast.

In this work we provide algorithms with analytical guarantees for combinatorial structures and time complexity. In particular, we formulate an optimization problem that takes as input a desired number of strings n and produces n length-ℓ strings that satisfy a specified set of constraints, while at the same time minimizing the length ℓ. We restrict our solution to this problem in two ways. First, we require that our algorithms run in time only polynomial in the number of strings n as well as any given constraint parameters. Second, we require that our algorithms produce sets of words that achieve word length ℓ that is within a constant multiple of the optimal achievable word length, while at the same time fulfilling the respective constraints with high probability. For various subsets of the constraints we propose, we provide algorithms that do this. We thus provide fast algorithms for the creation of sets of short words.

Paper Layout: In Section 2, we describe the different biologically motivated combinatorial constraints we use. In Section 3 we solve the design problem with subsets of constraints including the Hamming constraints, the consecutive bases constraint, and the GC content constraint. In Section 4 we extend our algorithms to deal with the free energy constraint.

2 Preliminaries

2.1 Notations

Let $X = x_1 x_2 \ldots x_\ell$ be a word where x_i belongs to some alphabet Π. In this paper we deal with two alphabets, namely, the binary alphabet $\Pi_B = \{0, 1\}$ and the DNA alphabet $\Pi_D = \{A,C,G,T\}$. The elements of an alphabet are called *characters*. We will use capital letters for words and small letters for characters. Our goal is to design DNA words but some of our algorithms generate binary words in intermediate steps.

The *reverse* of X, denoted by X^R, is the word $x_\ell x_{\ell-1} \ldots x_1$. The *complement* of a character x is denoted by x^c. The complements for the binary alphabet are given by $0^c = 1$, $1^c = O$, and for the DNA alphabet we have $A^c = T$, $C^c = G$, $G^c = C$, $T^c = A$.

The *complement* of a word is obtained by taking the complement of each of the characters in the word, i.e., $X^C = x_1^c x_2^c \ldots x_\ell^c$. The *reverse complement* of X is the complement of X^R, $X^{RC} = x_\ell^c x_{\ell-1}^c \ldots x_1^c$. The *Hamming distance* $H(X, Y)$ between two words X and Y is the number of positions where X differs from Y.

We are interested in designing a set $\mathcal{W}$ of n words over Π_D each of length ℓ which satisfy the constraints defined in Section 2.2 below.

2.2 Constraints

The constraints we consider can be classified into two categories: *non-interaction* constraints and *stability* constraints. Non-interaction constraints ensure that unwanted hybridizations between two DNA strands are avoided, and stability constraints ensure that the DNA strands are stable in a solution. The first six

constraints below are non-interaction constraints while the remaining three are stability constraints.

$C_1(k_1)$: **Basic Hamming Constraint** (k_1) = for any words $Y, X \in \mathcal{W}$, $H(Y, X) \geq k_1$.

This constraint limits non-specific hybridizations between the Watson-Crick complement of some word Y with a distinct word X.

$C_2(k_2)$: **Reverse Complementary Constraint** (k_2) = for any words $Y, X \in \mathcal{W}$, $H(Y, X^{RC}) \geq k_2$.

This constraint is intended to limit hybridization between a word and the reverse of another word.

$C_3(k_3)$: **Self Complementary Constraint** (k_3) = for any word Y, $H(Y, Y^{RC}) \geq k_3$.

This constraint prevents a word from hybridizing with itself.

$C_4(k_4)$: **Shifting Hamming Constraint** (k_4) = for any two words $Y, X \in \mathcal{W}$,

$$H(Y[1..i], X[(\ell - i + 1)..\ell]) \geq k_4 - (\ell - i) \text{ for all } i.$$

This is a stronger version of the Basic Hamming Constraint.

$C_5(k_5)$: **Shifting Reverse Complementary Constraint** (k_5) = for any two words $Y, X \in \mathcal{W}$,

$$H(Y[1..i], X[1..i]^{RC}) \geq k_5 - (\ell - i) \text{ for all } i; \text{and}$$

$$H(Y[(\ell - i + 1)..\ell], X[(\ell - i + 1)..\ell]^{RC}) \geq k_5 - (\ell - i) \text{ for all } i.$$

This is a stronger version of the Reverse Complementary Constraint.

$C_6(k_6)$: **Shifting Self Complementary Constraint** (k_6) = for any word $Y \in \mathcal{W}$,

$$H(Y[1..i], Y[1..i]^{RC}) \geq k_6 - (\ell - i) \text{ for all } i; \text{and}$$

$$H(Y[(\ell - i + 1)..\ell], Y[(\ell - i + 1)..\ell]^{RC}) \geq k_6 - (\ell - i) \text{ for all } i.$$

This is a stronger version of the Self Complementary Constraint.

$C_7(\gamma)$: **GC Content Constraint** (γ) = γ percentage of bases in any word $Y \in \mathcal{W}$ are either G or C.

The GC content affects the thermodynamic properties of a word [14–16]. Therefore, having the same ratio of GC content for all the words will assure similar thermodynamic characteristics.

$C_8(d)$: **Consecutive Base Constraint** (d) = no word has more than d consecutive bases for $d \geq 2$.

In some applications, consecutive occurrences (also known as runs) of the same base increase the number of annealing errors.

$C_9(\sigma)$: **Free Energy Constraint** (σ) = for any two words $Y, X \in \mathcal{W}$, $\text{FE}(Y) - \text{FE}(X) \leq \sigma$ where $\text{FE}(W)$ denotes the free energy of a word defined in Section 4.

This constraint ensures that all the words in the set have similar melting temperatures which allows hybridization of multiple DNA strands to proceed simultaneously [13].

For each of the given constraints above we assign a shorthand boolean function $C_i(t)$ to denote whether or not a given set of words $\mathcal{W}$ fulfills constraint C_i with respect to parameter t. For a given integer n, the goal of DNA word design is to efficiently create a set of n length-ℓ words such that a given subset of the above constraints are satisfied, while trying to minimize ℓ. That is, for a given subset of constraints $\{C_{\pi_1}, C_{\pi_2}, \ldots, C_{\pi_r}\} \subseteq \{C_1, C_2, \ldots, C_9\}$, the corresponding DNA word design (DWD) optimization problem is as follows.

*Problem 1 (*DWD$_{\pi_1, \pi_2, \ldots, \pi_r}$*).*
INPUT: Integers $n, t_1, t_2, \ldots, t_r$.
OUTPUT: A set $\mathcal{W}$ of n DNA strings each of the minimum length such that for all $1 \leq i \leq r$ the constraint $C_{\pi_i}(t_i)$ is satisfied over set $\mathcal{W}$.

For this problem we have the following trivial lower bounds for time complexity and the word size ℓ when any one of the first six constraints is applied.

Theorem 1. *Consider a set $\mathcal{W}$ of n DNA words each of length ℓ.*

1. *If $\mathcal{W}$ fulfills any one of the constraints $C_1(k), C_2(k), C_3(k), C_4(k), C_5(k)$, and $C_6(k)$, then $\ell = \Omega(k + \log n)$.*
2. *The time complexity of producing a set $\mathcal{W}$ that fulfills any one of the constraints $C_1(k)$, $C_2(k)$, $C_3(k)$, $C_4(k)$, $C_5(k)$, and $C_6(k)$ is $\Omega(nk + n \log n)$.*

The goal of DNA word design is to simultaneously satisfy as many of the above nine constraints as possible while achieving words within a constant factor of the optimal length ℓ for the given set of constraints. In Section 3 we show how to accomplish this goal for various subsets of the constraints.

3   Algorithms for DNA Word Design

In this section we develop randomized algorithms to generate sets of length-ℓ DNA words that satisfy certain sets of constraints while keeping ℓ within a constant of the optimal value. In particular, we first show how simply generating a set of n words at a specific length $\ell = O(k + \log n)$ uniformly at random is sufficient to fulfill constraints 1, 2, 3, 4, 5, and 6 simultaneously with high probability. We then propose three extensions to this algorithm to fulfill different subsets of constraints within a constant factor of the optimal word length. The first extension yields an algorithm for fulfilling the GC content constraint while the second yields one for the consecutive base and GC content constraints at the cost of the shifting constraints. Finally, we extend the basic randomized algorithm to fulfill the free energy constraint. The first is thus an algorithm for simultaneously fulfilling constraints 1, 2, 3, 4, 5, 6, and 7, the second simultaneously fulfills constraints 1, 2, 3, 7, and 8, and the last one fulfills constraints 1, 2, 3, 4, 5, 6 and 9.

Algorithm $FastDWD_{1,2,3,4,5,6}(n, k_1, k_2, k_3, k_4, k_5, k_6)$

1. Let $k = \max\{k_1, k_2, k_3, k_4, k_5, k_6\}$.
2. Generate a set $\mathcal{W}$ of n words over Π_D of length $\ell = 9 \cdot \max\{k, \lceil \log_4 n \rceil\}$ uniformly at random.
3. Output $\mathcal{W}$.

Fig. 1. A randomized algorithm for generating n DNA strings satisfying constraints $C_1(k_1)$, $C_2(k_2)$, $C_3(k_3)$, $C_4(k_4)$, $C_5(k_5)$, and $C_6(k_6)$.

3.1 A Simple Randomized Algorithm

*Problem 2 (*DWD$_{1,2,3,4,5,6}$*).*
INPUT: Integers n, k_1, k_2, k_3, k_4, k_5, k_6.
OUTPUT: A set $\mathcal{W}$ of n DNA strings each of the minimum length such that the constraints $C_1(k_1)$, $C_2(k_2)$, $C_3(k_3)$, $C_4(k_4)$, $C_5(k_5)$, $C_6(k_6)$ hold.

The next theorem shows that Algorithm FastDWD$_{1,2,3,4,5,6}$ $(n, k_1, k_2, k_3, k_4, k_5, k_6)$ in Figure 1 yields a polynomial-time solution to the $DWD_{1,2,3,4,5,6}$ problem with high probability. We omit the proof in the interest of space.

Theorem 2. *Algorithm* FastDWD$_{1,2,3,4,5,6}$ *produces a set $\mathcal{W}$ of n DNA words of optimal length $\Theta(k + \log n)$ in optimal time $\Theta(n \cdot k + n \cdot \log n)$ satisfying constraints $C_1(k_1)$, $C_2(k_2)$, $C_3(k_3)$, $C_4(k_4)$, $C_5(k_5)$ and $C_6(k_6)$ with probability of failure $o(1/(n + 4^k))$, where $k = \max\{k_1, k_2, k_3, k_4, k_5, k_6\}$.*

Proof (Sketch). The probability that two random words violate any of the constraints $C_1(k_1)$, $C_2(k_2)$, $C_4(k_4)$, and $C_5(k_5)$, can be bounded using Chernoff type bounds. Similarly, we can bound the probability of a random word violating any of the constraints $C_3(k_3)$ and $C_6(k_6)$.

We can then apply the Boole-Bonferroni Inequalties to yield a bound on the probability that any pair of words in a set of n random words violates constraints $C_1(k_1)$, $C_2(k_2)$, $C_4(k_4)$, or $C_5(k_5)$; or that any single word violates constraints $C_3(k_3)$ or $C_6(k_6)$. $\qquad\square$

3.2 Incorporating the GC Content Constraint into FastDWD$_{1,2,3,4,5,6}$

Now we show how to modify Algorithm FastDWD$_{1,2,3,4,5,6}$ so that it produces a set of words that also satisfies the GC content constraint. That is, we will show how to solve the following problem.

*Problem 3 (*DWD$_{1,2,3,4,5,6,7}$*).*
INPUT: Integers n, k_1, k_2, k_3, k_4, k_5, k_6, γ.
OUTPUT: A set $\mathcal{W}$ of n DNA strings each of the minimum length such that the constraints $C_1(k_1)$, $C_2(k_2)$, $C_3(k_3)$, $C_4(k_4)$, $C_5(k_5)$, $C_6(k_6)$, $C_7(\gamma)$ hold.

Algorithm $FastDWD_{1,2,3,4,5,6,7}(n, k_1, k_2, k_3, k_4, k_5, k_6, \gamma)$

1. Let $k = \max\{k_1, k_2, k_3, k_4, k_5, k_6\}$.
2. Generate a set $\mathcal{W}$ of n words over the binary alphabet Π_B of length $\ell = 10 \cdot \max\{k, \lceil \log_2 n \rceil\}$ uniformly at random.
3. For each word $W \in \mathcal{W}$, for any $\lceil \gamma \cdot \ell \rceil$ characters in W, replace 0 by G and 1 by C. For the remaining characters replace 0 by A and 1 by T to get W'. Let $\mathcal{W}'$ be the set of all words W'.
4. Output $\mathcal{W}'$.

Fig. 2. A randomized algorithm for generating n DNA strings satisfying constraints $C_1(k_1)$, $C_2(k_2)$, $C_3(k_3)$, $C_4(k_4)$, $C_5(k_5)$, $C_6(k_6)$, and $C_7(\gamma)$.

We modify Algorithm FastDWD$_{1,2,3,4,5,6}$ to get Algorithm FastDWD$_{1,2,3,4,5,6,7}$ shown in Figure 2. The next theorem shows that FastDWD$_{1,2,3,4,5,6,7}$ yields a polynomial-time solution to $DWD_{1,2,3,4,5,6,7}$ with high probability. We omit the proof in the interest of space.

Theorem 3. *Algorithm* FastDWD$_{1,2,3,4,5,6,7}$ *produces a set $\mathcal{W}$ of n DNA words of optimal length $\Theta(k + \log n)$ in optimal time $\Theta(n \cdot k + n \cdot \log n)$ satisfying constraints $C_1(k_1)$, $C_2(k_2)$, $C_3(k_3)$, $C_4(k_4)$, $C_5(k_5)$, $C_6(k_6)$, and $C_7(\gamma)$ with probability of failure $o(1/(n + 2^k))$, where $k = \max\{k_1, k_2, k_3, k_4, k_5, k_6\}$.*

3.3 Incorporating the Consecutive Bases Constraint into FastDWD$_{1,2,3,4,5,6,7}$

Now we modify Algorithm FastDWD$_{1,2,3,4,5,6,7}$ so that it produces a set that satisfies both the GC content constraint and the consecutive base constraint at the cost of the shifting constraints. That is, we will show how to solve the following problem.

*Problem 4 (*DWD$_{1,2,3,7,8}$*).*
INPUT: Integers n, k_1, k_2, k_3, γ, d.
OUTPUT: A set $\mathcal{W}$ of n DNA strings each of the minimum length such that the constraints $C_1(k_1)$, $C_2(k_2)$, $C_3(k_3)$, $C_7(\gamma)$, $C_8(d)$ hold.

We use Algorithm BreakRuns shown in Figure 3 to break long runs for a binary word so that it satisfies the consecutive bases constraint with parameter d. Intuitively what this algorithm does is for a given word X, it outputs X' by inserting characters at intervals of $d - 1$ from the left and the right in a manner such that there are no consecutive runs of length greater than d. We need to add characters from both ends to ensure that $H(X, Y^{RC}) \leq H(X', Y'^{RC})$ where X' and Y' are the respective outputs for X and Y from BreakRuns.

We modify Algorithm FastDWD$_{1,2,3,4,5,6,7}$ to get Algorithm FastDWD$_{1,2,3,7,8}$ shown in Figure 3. The next theorem shows that FastDWD$_{1,2,3,7,8}$ yields a polynomial-time solution to DWD$_{1,2,3,7,8}$ with high probability. We omit the proof in the interest of space.

Algorithm $BreakRuns(X, d)$

1. Let $X = x_1 x_2 \ldots x_\ell$. For $0 < i \le \lceil \frac{\ell}{2(d-1)} \rceil - 1$, let $x'_{\ell_i} = x^c_{i(d-1)}$ and $x'_{r_i} = x^c_{\ell - i(d-1)}$. Let $x'_{\text{mid}} = x^c_{\lfloor \ell/2 \rfloor}$.
2. Output $X' = x_1 \ldots x_{d-1} x'_{\ell_1} x_d \ldots x_{\lfloor \ell/2 \rfloor} x'_{\text{mid}} x_{\lfloor \ell/2 \rfloor + 1} \ldots x_{\ell-(d-1)-1} x'_{r_1} x_{\ell-(d-1)} \ldots x_\ell$.

Algorithm $\text{FastDWD}_{1,2,3,7,8}(n, k_1, k_2, k_3, \gamma, d)$

1. Let $k = \max\{k_1, k_2, k_3\}$.
2. Generate a set $\mathcal{W}$ of n words over the binary alphabet Π_B of length $\ell = 10 \cdot \max\{k, \lceil \log_2 n \rceil\}$ uniformly at random.
3. For each word $W \in \mathcal{W}$, let $W' = \text{BreakRuns}(W, d)$. Let $\mathcal{W}'$ be the set of all words W'.
4. For each word $W' \in \mathcal{W}'$, for any $\lceil \gamma \cdot \ell \rceil$ characters in W', replace 0 by G and 1 by C. For the remaining characters replace 0 by A and 1 by T to get W''. Let $\mathcal{W}''$ be the set of all words W''.
5. Output $\mathcal{W}''$.

Fig. 3. Algorithms for generating n DNA strings satisfying constraints $C_1(k_1)$, $C_2(k_2)$, $C_3(k_3)$, $C_7(\gamma)$, and $C_8(d)$.

Theorem 4. *Algorithm* $\text{FastDWD}_{1,2,3,7,8}$ *produces a set $\mathcal{W}$ of n DNA words of optimal length $\Theta(k + \log n)$ in optimal time $\Theta(n \cdot k + n \cdot \log n)$ satisfying constraints $C_1(k_1)$, $C_2(k_2)$, $C_3(k_3)$, $C_7(\gamma)$, and $C_8(d)$ with probability of failure $o(1/(n + 2^k))$, where $k = \max\{k_1, k_2, k_3\}$.*

4 Incorporating the Free Energy Constraint into FastDWD$_{1,2,3,4,5,6}$

Now we give an alternate modification of Algorithm $\text{FastDWD}_{1,2,3,4,5,6}$ such that the free energy constraint is satisfied. The free-energy $\text{FE}(X)$ of a DNA word $X = x_1 x_2 \ldots x_\ell$ is approximated by $\text{FE}(X) = \text{correction factor} + \sum_{i=1}^{\ell-1} \Gamma_{x_i, x_{i+1}}$, where $\Gamma_{x,y}$ is the pairwise free energy between base x and base y [7]. For simplicity, we denote the free energy as simply the sum $\sum_{i=1}^{\ell-1} \Gamma_{x_i, x_{i+1}}$ with respect to a given pairwise energy function Γ. Let $\Gamma_{\max}$ and $\Gamma_{\min}$ be the maximum and the minimum entries in Γ respectively. Let $D = \Gamma_{\max} - \Gamma_{\min}$.

We now show how to satisfy the free energy constraint $C_9(\sigma)$ for a constant $\sigma = 4D + \Gamma_{\max}$, while simultaneously satisfying constraints $1, 2, 3, 4, 5$, and 6. That is, we show how to solve the following problem.

Problem 5 ($\text{DWD}_{1,2,3,4,5,6,9}$).
INPUT: Integers n, k_1, k_2, k_3, k_4, k_5, k_6.
OUTPUT: A set $\mathcal{W}$ of n DNA strings each of the minimum length such that the constraints $C_1(k_1)$, $C_2(k_2)$, $C_3(k_3)$, $C_4(k_4)$, $C_5(k_5)$, $C_6(k_6)$, $C_9(4D + \Gamma_{\max})$ hold.

We modify Algorithm $\text{FastDWD}_{1,2,3,4,5,6}$ to get Algorithm $\text{FastDWD}_{1,2,3,4,5,6,9}$ shown in Figure 4 for solving $DWD_{1,2,3,4,5,6,9}$. The following lemmas identify the prop-

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Algorithm $FastDWD_{1,2,3,4,5,6,9}(n, k_1, k_2, k_3, k_4, k_5, k_6)$

Let $\hat{S}^1, \hat{S}^2, \ldots, \hat{S}^{4^m}$ be all possible sequences of length $m = 2\ell$ where ℓ is as defined in Step 2 below such that $\mathrm{FE}(\hat{S}^1) \leq \mathrm{FE}(\hat{S}^2) \leq \cdots \leq \mathrm{FE}(\hat{S}^{4^m})$. For two strings X and Y of respective lengths ℓ_X and ℓ_Y where ℓ_Y is even, let $X \otimes Y$ be the string $Y[1..(\ell_Y/2)]$ $X[1..\ell_X]$ $Y[(\ell_Y/2 + 1)..\ell_Y]$. Let $\Delta = \max_i\{\mathrm{FE}(\hat{S}^{i+1}) - \mathrm{FE}(\hat{S}^i)\}$.

1. Let $k = \max\{k_1, k_2, k_3, k_4, k_5, k_6\}$.
2. Generate a set $\mathcal{W}$ of n DNA words of length $\ell = 9 \cdot \max\{k, \lceil \log_4 n \rceil\}$ uniformly at random.
3. Let $\mathcal{W}_{\max} = \max_{X \in \mathcal{W}}\{\mathrm{FE}(X)\}$ and $\mathcal{W}_{\min} = \min_{X \in \mathcal{W}}\{\mathrm{FE}(X)\}$.
   if $\mathcal{W}_{\max} - \mathcal{W}_{\min} \leq 3D$, **then output** $\mathcal{W}$.
   else
4. Let $\alpha = \mathcal{W}_{\max} + \hat{S}^1$ and $\beta = \alpha + \Delta$. For each $S_i \in \mathcal{W}$, find $\hat{S}_j$ such that $\alpha \leq \mathrm{FE}(S_i) + \mathrm{FE}(\hat{S}_j) \leq \beta$. Let $W_i' = S_i \otimes \hat{S}_j$.
5. **output** $\mathcal{W}' = \{W_1', \ldots, W_n'\}$.

Fig. 4. A randomized algorithm for generating n DNA strings satisfying constraints $C_1(k_1)$, $C_2(k_2)$, $C_3(k_3)$, $C_4(k_4)$, $C_5(k_5)$, $C_6(k_6)$, and $C_9(4D + \Gamma_{\max})$.

erties of symbols $\Delta, \mathcal{W}, \mathcal{W}_{\max}, \mathcal{W}_{\min}, S_i, \hat{S}_j, \alpha, \beta$, and W_i' defined in Figure 4 and are used for proving the correctness of Algorithm FastDWD$_{1,2,3,4,5,6,9}$.

Lemma 1. $\Delta < 2D$.

Lemma 2. *If* $\mathcal{W}_{\max} - \mathcal{W}_{\min} > 3D$, *then* $\mathcal{W}_{\max} - \mathcal{W}_{\min} + 2D \leq \mathrm{FE}(\hat{S}^{4^m}) - \mathrm{FE}(\hat{S}^1)$.

Lemma 3. *For each* $S_i \in \mathcal{W}$, *there exists* $\hat{S}_j$ *such that* $\alpha \leq \mathrm{FE}(S_i) + \mathrm{FE}(\hat{S}_j) \leq \beta$.

Lemma 4. *For all* i, $\alpha - D \leq \mathrm{FE}(W_i') \leq \beta + D + \Gamma_{\max}$.

Section 4.1 discusses the details for Step 4 of the algorithm. Finally, Section 4.2 establishes its correctness and time complexity.

4.1 Computing Strings with Bounded Energies

In Step 4 of Algorithm FastDWD$_{1,2,3,4,5,6,9}$ we need to produce a set of n DNA strings $\hat{S}_1, \hat{S}_2, \ldots \hat{S}_n$, each of a given length $L = m$, such that $A_i \leq \mathrm{FE}(\hat{S}_i) \leq B_i$ for some A_i, B_i such that $B_i - A_i \leq \Delta$. That is, we need to solve the following problem.

Problem 6 (Bounded-Energy Strand Generation).
INPUT:

1. Integers A_i and B_i for $i = 1$ to n such that
   (a) $A_i \geq \mathcal{W}_{\min}$;
   (b) $B_i \leq \mathcal{W}_{\max}$;
   (c) $B_i - A_i \leq \Delta$.
2. Length L.

OUTPUT: Strings $\hat{S}_1, \hat{S}_2, \ldots \hat{S}_n$ each of length L and respective energy E_i such that $A_i \leq E_i \leq B_i$.

Algorithm $ConstructStrings(\{A_i\}, \{B_i\}, L)$

1. Let $\Phi \leftarrow \text{Build}(L)$.
2. **if** $n \geq \sqrt{\frac{L}{\log L}}$, **then** $\Psi \leftarrow \text{SlowBuild}(L)$, **else** $\Psi \leftarrow \text{NULL}$.
3. For each $i = 1$ to n, find a nonzero coefficient ζ_{E_i} of X^{E_i} in some polynomial $f_L^{a,b}(x) \in \Phi$ such that $A_i \leq E_i \leq B_i$.
4. For $i = 1$ to n, set $\hat{S}_i = \text{Extract}(E_i, \Phi, \Psi)$.

Fig. 5. This algorithm solves the Bounded Energy Strand Generation Problem (Problem 6).

Our solution to this problem involves transforming the blunt of the computational task into the problem of polynomial multiplication. Consider the following polynomial.

Definition 1. *For any integer $\ell \geq 1$, let $f_{\ell,a,b}(x)$ be the polynomial $\sum_{z=0}^{\ell \cdot m} \zeta_z x^z$ where coefficient ζ_z is the number of length-ℓ strings whose first character is a, last character is b, and free energy is z.*

For $f_\ell(x) = \sum_{\forall a,b \in \Pi} f_{\ell,a,b}(x)$ the coefficient of x^i denotes the number of strings of length ℓ and free energy i. As a first step towards our solution, we use a subroutine BUILD(L) which computes Φ, the polynomials $f_{L,a,b}(x)$, $f_{\lfloor L/2 \rfloor,a,b}(x), \ldots, f_{1,a,b}(x)$, for all $a, b \in \Pi$ in $O(L \log L)$ time. The efficient computation of these polynomials relies on the following recursive property.

Lemma 5. *For any integers $\ell_1, \ell_2 \geq 1$,*

$$f_{\ell_1 + \ell_2, a, b}(x) = \sum_{d_1, d_2 \in \Pi} f_{\ell_1, a, d_1}(x) \cdot f_{\ell_2, d_2, b}(x) \cdot x^{\Gamma_{d_1, d_2}}.$$

The problem of determining the number of strings of length L and free energy E is considered in [12] and a dynamic programming based $O(L^2)$-time algorithm is provided. However, exploiting the recursive property of Lemma 5 and Fast Fourier Transforms [8] for polynomial multiplication the subroutine BUILD solves this problem in faster $O(L \log L)$ time and may be of independent interest.

Our algorithm for Problem 6 has two phases, the *build* phase and the *extract* phase. The build phase constructs a data structure that permits the extract phase to be executed quickly. In the extract phase, an extraction routine is run n times to output $\hat{S}_i$ for each $i \in [1, n]$. Since the extraction routine is executed n times and the build routine only once, the phase that constitutes the bottleneck for our algorithm for Problem 6 depends on the values of n and L. We thus provide two forks for the algorithm to take, one with a fast build routine and a modestly fast extract routine, and the other with a slower build routine but an optimally fast extract routine. In particular, if n is sufficiently larger than L, our algorithm for Problem 6 calls a routine SlowBuild(L) which improves the runtime of Extract. Otherwise, only a faster BUILD function is called in the first phase, leading to a slower Extract routine. The algorithm for Problem 6 is given in Figure 5.

Algorithm ConstructStrings makes use of three subroutines – Build, SlowBuild and Extract. The procedure Build(L) computes Φ, a data structure containing for all $a, b \in \Pi$ and a given L, the polynomials $f_{\ell,a,b}(x)$ for $\ell = L, \lfloor \frac{L}{2} \rfloor, \lfloor \frac{L}{4} \rfloor, \lfloor \frac{L}{8} \rfloor, \ldots, 1$. This permits Extract($E, \Phi, \Psi$) to obtain a length L string of energy E in time $O(L \log L)$.

A call to SlowBuild(L) of time complexity $O(L^{1.5} \log^{0.5} L)$ improves the complexity of Extract(E, Φ, Ψ) to $O(L)$ by computing Ψ, a data structure containing for every non-zero term x^i in $f_{\lfloor \frac{L}{2^a} \rfloor, a, b}$ a corresponding pair of non-zero terms x^j and $x^{i-j-\Gamma_{d_1, d_2}}$ in $f_{\lfloor \frac{L}{2^{a+1}} \rfloor, a, d_1}$ and $f_{\lfloor \frac{L}{2^{a+1}} \rfloor, d_2, b}$ respectively. This yields the following theorem.

Theorem 5. *Algorithm* ConstructStrings($\{A_i\}, \{B_i\}, L$) *solves Problem 6 in time* $O(\min\{nL \log L, L^{1.5} \log^{0.5} L + nL\})$.

4.2 Putting it all together for DWD$_{1,2,3,4,5,6,9}$

Theorem 6. *Algorithm* FastDWD$_{1,2,3,4,5,6,9}$ *produces a set of n DNA words of optimal length $\Theta(k + \log n)$ in time $O(\min\{n\ell \log \ell, \ell^{1.5} \log^{0.5} \ell + n\ell\})$ satisfying the constraints $C_1(k_1)$, $C_2(k_2)$, $C_3(k_3)$, $C_4(k_4)$, $C_5(k_5)$, $C_6(k_6)$, and $C_9(4D + \Gamma_{\max})$ with probability of failure $o(1/(n + 4^k))$, where $k = \max\{k_1, k_2, k_3, k_4, k_5, k_6\}$.*

Proof. From Theorem 2 we know that $\mathcal{W}$ satisfies constraints $C_1(k_1)$, $C_2(k_2)$, $C_3(k_3)$, $C_4(k_4)$, $C_5(k_5)$, and $C_6(k_6)$ with probability of failure $o(1/(n + 4^k))$. If $\mathcal{W}_{\max} - \mathcal{W}_{\min} \leq 3D$, then FastDWD$_{1,2,3,4,5,6,9}$ outputs $\mathcal{W}$ which satisfies $C_9(3D)$ and hence also satisfies $C_9(4D + \Gamma_{\max})$. Otherwise, it is easy to verify that since $\mathcal{W}$ satisfies these six constraints, so does $\mathcal{W}'$. From Lemma 3 we know that there always exists a string $\hat{S}_j$ as required in Step 4 of FastDWD$_{1,2,3,4,5,6,9}$. Further, Lemma 4 shows that $\mathcal{W}'$ satisfies $C_9(\Delta + 2D + \Gamma_{\max})$. Therefore, $\mathcal{W}'$ satisfies constraints $C_1(k_1)$, $C_2(k_2)$, $C_3(k_3)$, $C_4(k_4)$, $C_5(k_5)$, $C_6(k_6)$, and $C_9(4D + \Gamma_{\max})$ with the stated failure probability.

The length of any word $W' \in \mathcal{W}'$ is at most 3ℓ where $\ell = \Theta(k + \log n)$, which is optimal from Theorem 1.

Generating $\mathcal{W}$ takes $O(n \cdot k + n \cdot \log n)$ time. The bulk of the time complexity for the algorithm comes from Step 4, which is analyzed in Section 4.1 to get $O(\min\{nL \log L, L^{1.5} \log^{0.5} L + nL\})$ (see Theorem 5) where $L = O(\ell)$. $\qquad \square$

5 Future Work

A number of problems related to this work remain open. It is still unknown how to generate words of optimal length that simultaneously satisfy the free energy constraint and the consecutive bases constraint. We also have not provided a method for combining the consecutive bases constraint with any of the shifting constraints.

Another open research area is the verification problem of testing whether or not a set of words satisfy a given set of constraints. This problem is important because our algorithms only provide a high-probability assurance of success. While verification can clearly be done in polynomial time for all of our constraints, the naive method of verification has a longer runtime than our algorithms for constructing the sets. Finding faster, non-trivial verification algorithms is an open problem.

A third direction for future work involves considering a generalized form of the basic Hamming constraint. There are applications in which it is desirable to design sets of words such that some distinct pairs bind with one another, while others do not [2, 14]. In this scenario, we can formulate a word design problem that takes as input a matrix of pairwise requirements for Hamming distances. Determining when such a problem is solvable and how to solve it optimally when it is are open problems.

References

[1] L. M. ADLEMAN, *Molecular Computation of Solutions to Combinatorial Problems*, Science, 266 (1994), pp. 1021–1024.

[2] G. AGGARWAL, M. H. GOLDWASSER, M.-Y. KAO, AND R. T. SCHWELLER, *Complexities for Generalized Models of Self-Assembly*, in Proceedings of the 15th Annual ACM-SIAM Symposium on Discrete Algorithms, 2004, pp. 880–889.

[3] A. BEN-DOR, R. KARP, B. SCHIWKOWSKI, AND Z. YAKHINI, *Universal DNA Tag Systems: A Combinatorial Design Scheme*, in Proceedings of the 4th Annual International Conference on Computational Molecular Biology, 2000, pp. 65–75.

[4] S. BRENNER, *Methods for Sorting Polynucleotides using Oligonucleotide Tags*. US Patent Number 5,604,097, February 1997.

[5] S. BRENNER AND R. A. LERNER, *Encoded Combinatorial Chemistry*, in Proceedings of Natianal Academy of Science, vol. 89, June 1992, pp. 5381–5383.

[6] A. BRENNERMAN AND A. E. CONDON, *Strand Design for Bio-Molecular Computation*, Theoretical Computer Science, 287 (2001), pp. 39–58.

[7] K. J. BRESLAUER, R. FRANK, H. BLOCKER, AND L. A. MARKY, *Predicting DNA Duplex Stability from the Base Sequence* , in Proceedings of the National Academy of Sciences, vol. 83, 1986, pp. 3746–3750.

[8] T. H. CORMEN, C. L. LEISERSON, R. L. RIVEST, AND C. STEIN, *Introduction to Algorithms*, MIT Press, Cambridge, MA, 2nd ed., 2001.

[9] R. DEATON, M. GARZON, R. MURPHY, D. FRANCESCHETTI, AND S. STEVENS, *Genetic Search of Reliable Encodings for DNA Based Computation*, in Proceedings of the 1st Annual Conference on Genetic Programming, 1996, pp. 9–15.

[10] A. G. FRUTOS, Q. LIU, A. J. THIEL, A. M. W. SANNER, A. E. CONDON, L. M. SMITH, AND R. M. CORN, *Demonstration of a Word Design Strategy for DNA Computing on Surfaces*, Nucleic Acids Research, 25 (1997), pp. 4748–4757.

[11] M. GARZON, R. DEATON, P. NEATHERY, D. FRANCESCHETTI, AND R. MURPHY, *A New Metric for DNA Computing*, in Proceedings of the 2nd Genetic Programming Conference, 1997, pp. 472–278.

[12] A. MARATHE, A. CONDON, AND R. M. CORN, *On Combinatorial DNA Word Design*, Journal of Computational Biology, 8 (2001), pp. 201–219.

[13] D. D. SHOEMAKER, D. A. LASHKARI, D. MORRIS, M. MITTMANN, AND R. W. DAVIS, *Quantitative Phenotypic Analysis of Yeast Deletion Mutants Using a Highly Parallel Molecular Bar-coding Strategy*, Nature, 16 (1996), pp. 450–456.

[14] S. A. TSAFTARIS, *DNA Computing from a Signal Processing Viewpoint*, IEEE Signal Processing Magazine, 21 (2004), pp. 100–106.

[15] D. C. TULPAN AND H. H. HOOS, *Hybrid Randomised Neighbourhoods Improve Stochastic Local Search for DNA Code Design*, in Lecture Notes in Computer Science 2671: Proceedings of the 16th Conference of the Canadian Society for Computational Studies of Intelligence, Y. Xiang and B. Chaib-draa, eds., Springer-Verlag, New York, NY, 2003, pp. 418–433.

[16] D. C. TULPAN, H. H. HOOS, AND A. CONDON, *Stochastic Local Search Algorithms for DNA Word Design*, in Lecture Notes in Computer Science 2568: Proceedings of the 8th International Workshop on DNA-Based Computers, M. Hagiya and A. Ohuchi, eds., Springer-Verlag, New York, NY, 2003, pp. 229–241.

[17] E. WINFREE, F. LIU, L. WENZLER, AND N. SEEMAN, *Design and Self-Assembly of Two-Dimensional DNA Crystals*, Nature, 394 (1998), pp. 539–544.