SPIDER-WEB enables stable, repairable, and encrytable algorithms under arbitrary local biochemical constraints in DNA-based storage

HAOLING ZHANG, BGI-Shenzhen, China
HAOLING ZHANG, BGI-Shenzhen, China
HZHAOJUN LAN, Capital Normal University, China
WENWEI ZHANG, BGI-Shenzhen, China
XUN XI, BGI-Shenzhen, China
ZHI PING, BGI-Shenzhen, China
YIWEI ZHANG, Shandong University, China
YUE SHEN, BGI-Shenzhen, China

DNA has been considered as a promising medium for storing digital information. Despite the biochemical progress in DNA synthesis and sequencing, novel coding algorithms need to be constructed under the specific constraints in DNA-based storage. Many functional operations and storage carriers were introduced in recent years, bringing in various biochemical constraints including but not confined to long single-nucleotide repeats and abnormal GC content. Existing coding algorithms are not applicable or unstable due to more local biochemical constraints and their combinations. In this paper, we design a graph-based architecture, named SPIDER-WEB, to generate corresponding graph-based algorithms under arbitrary local biochemical constraints. These generated coding algorithms could be used to encode arbitrary digital data as DNA sequences directly or served as a benchmark for the follow-up construction of coding algorithms. To further consider recovery and security issues existing in the storage field, it also provides pluggable algorithmic patches based on the generated coding algorithms: path-based correcting and mapping shuffling. They provide approaches for probabilistic error correction and symmetric encryption respectively.

Keywords: DNA-based storage, algorithm generation, probabilistic error correction, symmetric encryption.

1 INTRODUCTION

The total amount of data increases exponentially with the rapid development of human society [18]. DNA molecules, as a candidate for storage medium with great potential [14, 33], have drawn much attention for the incredibly storage density. The basic principle of DNA-based storage is the transformation between digital data (represented by binary messages) and DNA molecules (represented by DNA strings in silico). Thus, coding algorithm is one of the most basic but significant steps in DNA-based storage.

The most essential biochemical techniques in DNA-based storage are DNA synthesis (“writing” information) [30], polymerase chain reaction (PCR) amplification (“copying” information) [13], and DNA sequencing (“reading” information) [61]. Due to the limitation of these technologies, long single-nucleotide repeats (or homopolymer) in DNA molecules may cause difficulties or higher error rates in DNA synthesis and sequencing [24]. Some early coding algorithms [8, 10, 14, 24, 25] therefore limited the maximum length of homopolymer in encoded DNA strings by their customized mapping relationship such as rotating encoding [9]. To improve the stability of DNA sequencing coverage [53], the constraint of global GC content was introduced. Considering

Haoling Zhang and Zhaojun Lan contributed equally to this work; Yiwei Zhang and Yue Shen supervised jointly this study.

Authors’ addresses: Haoling Zhang, zhanghaoling@genomics.cn, BGI-Shenzhen, Shenzhen, Guangdong, China, 518120; Zhaojun Lan, zjl@cnu.edu.cn, Capital Normal University, Beijing, China, 100089; Wenwei Zhang, zhangww@genomics.cn, BGI-Shenzhen, Shenzhen, Guangdong, China, 518120; Xun Xu, xuxun@genomics.cn, BGI-Shenzhen, Shenzhen, Guangdong, China, 518120; Zhi Ping, pingzhi@genomics.cn, BGI-Shenzhen, Shenzhen, Guangdong, China, 518120; Yiwei Zhang, ywzhang@sdu.edu.cn, Shandong University, Qingdao, Shandong, China, 266237; Yue Shen, shenyue@genomics.cn, BGI-Shenzhen, Shenzhen, Guangdong, China, 518120.
the impact of these two constraints, some coding algorithms [42, 65] approached solutions while maintaining a relatively high code rate from binary messages to DNA strings.

Although the investigation of the algorithm design of the above biochemical constraints becomes deeper, these simple constraints cannot adapt to the development of the storage field. A few complex biochemical operations were introduced in DNA-based storage for more functions such as picture preview [60]. Some complex operations like DNA strand displacement [64], DNA hybridization [6], and transcription [35] are used to provide operability and functionality for DNA molecules. Meanwhile, considering the convenience of cell-line passaging and operation via molecular biological techniques, storing information in living cells is becoming a promising direction of DNA-based storage, which requests more potential constraints [56, 58]. These advanced operations bring additional biochemical constraints (see Background), making many established algorithms unsuitable. Providing coding algorithms case-by-case for these potential requirements is difficult and time-consuming. Hence, it would be increasingly important to create coding algorithm for arbitrary biochemical constraints while making its code rate approach capacity. HEDGES [50] attempted to contain various local biochemical constraints through modular operation to achieve this objective. However, without pretreatment (see Methodology), DNA strings may not be continually encoded under potential strict constraints. Besides, based on the validity screening procedure, which can only generate sequences satisfying corresponding requests, DNA Fountain [17] and Yin-Yang Code [47] tried to provide another perspective to deal with arbitrary constraints. Nevertheless, affected by byte frequency distribution [34] of processed digital data and specific parameter settings of the algorithm itself (see Evaluation), the code rate of these coding algorithms fluctuates (a few data patterns are uncodable) [48]. With these potential defects of the strategies mentioned above, it is challenging to design more robust high-performance algorithms.

Further, as the uncertainty [70] of biochemical operations bring difficulties for benchmarking, investigations of algorithm could be more convenient to calibrate some common issues, such as data recovery and security. Since DNA molecule error rate is much higher than that in hard disk (see Background), established coding algorithms introduced error-correcting code to increase the recovery success rate [17, 25, 47]. Nevertheless, no well-established error-correcting codes that have been strictly proved to be able to repair errors in DNA strings, especially for insertion and deletion [50].

With increasingly important role playing by DNA-based storage, it is predictable that privacy, or even security of information will gradually become a concern. Although at the arithmetic level, replacing the mapping rule in the coding algorithm is a typical procedure [40], such modification may make DNA strings to violate the established constraints or affect the code rate. Hence, developing a reliable encryption system for DNA-based storage is also necessary, which requires further investigation.

Table 1. Glossary of terms in this work

| item           | explanation                                                                                                                                 |
|----------------|---------------------------------------------------------------------------------------------------------------------------------------------|
| coding algorithm | conversion method between binary messages and DNA strings.                                                                                   |
| binary message  | sequence consisting of bit symbols “0” and “1”.                                                                                               |
| DNA string      | sequence consisting of nucleotide characters “A”, “C”, “G”, and “T”.                                                                          |
| code rate       | average number of bits per nucleotide encoded by a coding algorithm.                                                                           |
| capacity        | upper bound on the code rate at which binary messages can be encoded under requirements.                                                      |
| coding scenarios| parameter set constituted by binary message patterns (e.g. byte frequency distribution), biochemical constraints, and algorithm parameter settings. |
| uncodable       | selected coding algorithm cannot complete the encoding task (such as falling into an infinite loop [66]) under specific coding scenarios.        |
| unstable        | coding scenarios significantly affect the code rate of the selected coding algorithm.                                                        |
In this work, we established a general graph-based architecture, namely **SPIDER-WEB**. Under arbitrary local biochemical constraint(s), it could construct applicable coding algorithms automatically, with the code rate of constructed coding algorithms close to the capacity. Furthermore, it provides pluggable tools for probabilistic error correction and symmetric encryption under the generated coding algorithms.

2 BACKGROUND

2.1 increasingly biochemical constraints for functional operations and storage carriers

Compared with the operations of magnetic head on disk, biochemical operations for DNA molecules are not that robust. Errors in DNA-based storage are mainly caused by the limitation of current techniques (usually come from synthesis and sequencing, namely the basic pipeline in this work). Take the Illumina sequencing platform as an example, the average error rate determined was $0.24 \pm 0.06\%$ per nucleotide [46]. In contrast, it was reported that hard drives have a nominal annual failure rate of at most $0.88\%$ [55]. Therefore, to improve the compatibility for the basic pipeline (Figure 1), two kinds of well-accepted biochemical constraints have been widely used for encoding design: limited homopolymer run-length and global balanced GC content.

![Figure 1. Pipelines and operations in DNA-based storage](image-url)

It includes basic reading/writing processes, common storage carriers, and recent functional operations. Some functional operations, marking with experimental gloves, are not mature processes or without the support of automatic instruments.

To ensure the compatibility of more biochemical techniques, some novel constraints are beginning to be studied. Focusing on the "copying" process, some works [22, 41, 50] explored the regionalized GC content constraint to improve the success rate of PCR amplification techniques [7]. For the "reading" process, it has been reported that Illumina sequencing platforms (high-throughput sequencing) have high error rates following a "GGC" motif [10]. Meanwhile, aggregation of purines (A/G) or pyrimidines (C/T) may interfere with the sequencing results and cause retrieval errors in Nanopore techniques (single-molecule sequencing) [15]. Inspired by thermodynamically
driven interactions between DNA molecules, molecular-level similarity search [6, 60] provides innovative file operations while introducing constraints based on sequence difference.

Compared with in vitro storage, the requirements of saving in living cells (called the host) are more complex. GC content of inserted DNA molecules should be adapted to the GC content range of the host genome, which displays a wide range of variation. If the GC content of DNA molecules in endosymbiotic bacteria is between 10% and 30%, these bacteria show a higher fitness in natural selection [16]. It implies that AT-rich DNA molecules may contribute to the massive data distribution [12]. On the contrary, the molecules with high GC content (50% ~ 70%) and the repair of double-strand breaks are highly correlated in prokaryotes [67]. Hence, information with GC-rich may be stored longer in this type of host. Furthermore, some motifs are related to the life cycle of hosts, affecting the inserted DNA molecules. As a type of common motif, the restriction site [4] can be identified and bound by the specific restriction enzymes, like “GAATTC” for enzyme “EcoRI” [49]. DNA molecule with restriction site(s) has a high probability of being cleaved into pieces, resulting in the loss of large-scale information [36]. Further, some functional motifs, such as promoters [26], may also affect the hosts’ gene expression level.

With the requirement of more functions to be incorporated into DNA-based storage system, more biochemical constraints operability and functionality may be put forward. Some biochemical constraints may be difficult to define and formulize explicitly. Meanwhile, designing coding algorithms for these constraints and their combinations is time-consuming. Therefore, it is worth establishing an automaton to develop coding algorithms.

2.2 error correction to combat uncertainty of biochemical operations

Since biochemical operations are difficult to be conducted accurately at the single molecular level, applying biochemical constraints only cannot avoid the uncertainty during experiments [70]. To restore original information accurately, the fundamental strategy is to add excessive copies directly, such as alternate fragments [24] and XOR parity strand [10]. Many researchers have noticed the value of error-correcting codes for DNA-based storage to reduce redundancy and correct complex errors.

Achieving the goal of reducing redundancy is simple, many works [2, 17, 25, 43, 47, 50] introduced Reed-Solomon code [51]. In addition, due to the linear decoding complexity and Shannon limit error performance, low-density parity check codes [23] are also often considered [11, 12]. These well-established error-correcting codes provide an effective way to solve one or more substitution errors in DNA molecules. Insertion and deletion of one or more nucleotides in a DNA molecule will cause a “frameshift” and conventional error-correcting codes cannot detect where and how the errors occur. It is reported that some asymptotic optimal codes can correct one insertion or deletion error [32, 59]. Several advanced codes can correct multiple errors [44, 57], the redundancy of which is at least twice of asymptotic optimum. Further, these codes have additional requirements like knowing the length of the DNA string. Therefore, a probabilistic error correcting code has become a transitional strategy [50, 71] to provide solutions in practice.

3 METHODOLOGY

SPIDER-WEB is a graph-based architecture, which provides tools for creating stable, repairable, and encryptible algorithms under arbitrary local biochemical constraints. First of all, as a generator (Figure 2A), SPIDER-WEB provides graph-based coding algorithms under arbitrary local biochemical constraint combinations. Afterward, as a corrector (Figure 2B), when one or more edit (including insertion, deletion, or substitution) errors occur during the DNA sequencing process, SPIDER-WEB supplies a probabilistic repair strategy. Finally, as a confounder (Figure 2C), SPIDER-WEB offers specific encryption capabilities against eavesdropping.

To explain the following processes, we first introduce some graph-theoretic terminologies. A digraph \( \mathcal{D} = (\mathcal{V}, \mathcal{A}) \) consists of a set of vertices \( \mathcal{V} \) and a set of arcs (or directed edges) \( \mathcal{A} \). Each arc from the vertex \( u \) to the vertex \( v \) is denoted as \((u, v)\), where \( u \) is called the initial vertex and \( v \) is called the terminal of the arc \((u, v)\). The
number of arcs with $u$ as initial vertex is called the **out-degree of $u$, denoted as $\text{deg}^{+}_D(u)$. In DNA-based storage we always consider the quaternary alphabet \{A,C,G,T\}. Given positive integers $\ell$, the **4-ary de Bruijn graph of order $\ell$, denoted as $D^{\ell}_4$, is a digraph whose vertex set is $V = \{A,C,G,T\}^{\ell}$. For any two vertices $u = (u[1], u[2], \ldots, u[\ell])$ and $v = (v[1], v[2], \ldots, v[\ell])$, there is an **outgoing arc $(u, v)$ of $u$ in $D^{\ell}_4$ if and only if $u[i + 1] = v[i]$ for $1 \leq i \leq \ell - 1$, which is shown in Figure 3A. It is routine to check that every vertex in $D^{\ell}_4$ has out-degree 4.

\footnote{$v[i]$ refers to $i$-th nucleotide of $v$.}
3.1 graph-based encoding

The constraints considered in this paper include the homopolymer run-length constraints, the regionalized GC-content constraints, and occasionally a third kind of constraint forbidding undesired motifs (mathematical formulations of these constraints are listed in Appendix A1). Following a standard approach in constrained coding theory, we consider a state-transition digraph $D^C_\ell$, which is a subgraph of the de Bruijn graph $D^T_\ell$ induced by the vertices which satisfy the constraint set $C$. The digraph $D^C_\ell$ characterizes the constraints in the sense that every quaternary codeword satisfying the constraints can be represented as a directed path in $D^C_\ell$, and vice versa.

In SPIDER-WEB, the initialization step is to build an underlying digraph $D$ for further implementations. The detailed process is shown in Algorithm 1. The algorithm starts with a screening process by deleting vertices which correspond to sub-strings of length $\ell$ violating one or more constraints from $C$. After obtaining the digraph $D^C_\ell$ in step 1, it goes on to several recursive steps to further trim the vertex set, guaranteeing that the final output $D$ has minimum out-degree at least 2. The motivation behind these trimming steps is that vertices with out-degree 0 or 1 will either make the algorithm afterwards invalid [50] or affect the code rate (Figure S1).

**Algorithm 1:** Initializing step to build $D$

**Input:** de Bruijn graph $D^T_\ell$ and constraint set $C$.

**Output:** digraph $D$ satisfying considered constraints $C$ and the out-degree requirement.

1. Set $D^C_\ell$ through deleting all the vertices from $D^T_\ell$ which violate one or more constraints from $C$.
2. Set $D$ as a digraph induced by the vertex set of $D^C_\ell$.
3. Check the out-degree of each vertex in $D$.
4. If all vertices have out-degree at least 2, jump to step 6; otherwise, go to step 5.
5. Delete all the vertices with out-degree less than 2, then jump to step 3.
6. Output $D$.

After constructing $D$, we move onto a binding process, which binds each arc of $D$ with a digit. For each arc in $D$ from $u$ to $v$, where $u[1 : \ell - 1] = v[2 : \ell]$, we call it a $v[\ell]$-arc, $v[\ell] \in \{A, C, G, T\}$.

Each vertex has at most $\epsilon \leq 4$ outgoing arcs with distinct labels and we bind these arcs with digits $\{0, 1, \ldots, \epsilon - 1\}$ according to a predetermined partial order $A < C < G < T$, as the left table of Figure 2C. For example, if a vertex has three outgoing arcs with labels (like $\{A, C, T\}$), then (counting from zero) the A-arc is the 0-th one, the C-arc is the 1-st and the T-arc is the 2-nd. These arcs are further labeled to as $\{A|0\}$-arc, $\{C|1\}$-arc, and $\{T|2\}$-arc, called label arc.

After obtaining label arcs, $D$ will lead to a graph-based encoding algorithm $E$ as shown in Algorithm 2, which encodes $x$, a binary message of length $k$, into a DNA string

$$y = E(x),$$

and guarantees that $y$ satisfies the constraints $C$.

In Figure 2A, a piece of information has undergone three transformations from a binary message $x$ to a DNA string $y$. Initially, the binary message $[101010]$ is converted into the decimal number $42 = 2^{6-1} + 2^{6-3} + 2^{6-5}$. Such a decimal number is further disassembled into a graph-based vector through $D$. The value in each position of this vector depends on the out-degree of the vertex currently being accessed. As an example, the out-degree of virtual vertex is 4, the graph-based vector can obtain 2 in the first cycle because $42 \div 4 = 10 \cdots 2$ and the decimal number becomes 10. The next cycle could be $10 \div 2 = 5 \cdots 0$. The cycle ends until the decimal number becomes 0 and we obtain a graph-based vector [2012]. Based on $D$, [2012] is equivalent to the DNA string [GACT].

For simplicity, $u[i + 1] = v[i]$ for $1 \leq i \leq \ell - 1$" can be abbreviated as $u[2 : \ell] = v[1 : \ell - 1]$. To facilitate the understanding of more researchers, vector/matrix indices start at $1$ (rather than $0$ in modern programming languages).

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2 For simplicity, "$u[i + 1] = v[i]$ for $1 \leq i \leq \ell - 1$" can be abbreviated as $u[2 : \ell] = v[1 : \ell - 1]$. To facilitate the understanding of more researchers, vector/matrix indices start at $1$ (rather than $0$ in modern programming languages).
Algorithm 2: Encoding binary message through graph-based coding algorithm

Input: binary message \( x \).
Output: DNA string \( y \) satisfying considered constraints \( C \).

1. Convert \( x \) into the decimal number \( \pi \).
2. Pick a working vertex in \( D \) virtually \([24, 47]\) and set \( y \) as an empty string.
3. If \( \pi \) is greater than 0, go to step 4; otherwise, jump to step 8.
4. Set \( p \) to be the out-degree of the working vertex.
5. Divide \( \pi \) by \( p \) and let the remainder be \( r \) and the quotient be \( \lfloor \pi/p \rfloor \).
6. Find the \( \{*|r\} \)-arc and add \( * \in \{A,C,G,T\} \) to the end of \( y \).
7. Set \( \pi \) as \( \lfloor \pi/p \rfloor \) and set the working vertex as the terminal of the \( \{*|r\} \)-arc, then jump to step 3.
8. Output \( y \).

Reversely, in the decoding process, the DNA string will be first converted to the corresponding graph-based vector and then to the equivalent decimal number through the out-degree information of \( D \), and finally to the corresponding binary message through number-base conversion based on this decimal number and the known length of this binary message.

3.2 path-based correcting

Probabilistically, DNA strings obtained from the “reading” process may contain one or more errors, where the error type could be insertions, deletions, or substitutions. Many works on error-correcting codes against one type of error or a combination of two types of error have been done by coding theorists. However, if we consider the combination of all three types (which is referred to as an edit error), up till now there are only codes against one edit error. Constructing codes against multiple edit errors is still a challenging task in coding theory.

At first sight, it is tempting to directly learn from some known codes against one edit error into our SPIDER-WEB framework for error correction. Nevertheless, there are two disadvantages. Firstly, a proportion of the codewords may not satisfy the established constraint set and thus cannot be used. Secondly, when multiple errors occur (which is quite common), the decoding process for codes against only one edit error will no longer help.

Due to the structure of \( D \), our encoding scheme naturally brings some robustness against errors in the following sense. If there is no error then the output of our coding algorithm must be a directed path on \( D \), which is shown in Figure 3A. Otherwise, when errors occur then with high probability the output erroneous string will no longer be a valid directed path on \( D \). Therefore, we may check the validity of an output string by tracing the directed path on \( D \). Whenever we are at some vertex in \( D \) but fail to find the suitable label arc corresponding to the next nucleotide of the string, then we know that the nucleotide of current and/or previous positions is wrong. Thus, such errors can be detected timely (Figure 3B).

Once an error is spotted at the \( j \)-th position, we apply a saturated reverse search method for error correction (Figure 3C). Here “reverse” means that we check backward from the \( j \)-th to the \((j-\ell)\)-th position (in some sense according to a decreasing order of the error probability). When checking a particular position \( i \), \( j-\ell \leq i \leq j \), we guess the type of error and try these 3 adjustment types:

- substitute the nucleotide in its current position with another nucleotide;
- insert a nucleotide between \((i-1)\)-th position and \( i \)-th position;
- delete the nucleotide in \( i \)-th position.

Conservatively, all adjustment types need to be tested in each position of the local range (which accounts for “saturated”). The number of adjustments includes at most 3 substitutions, 4 insertions and 1 deletion. The precise
**Case of path-based correcting.** (A) creates a GC-balanced digraph of length 2. Through such digraph, the binary message \([001101]\) is encoded to the DNA string \([TCTGAC]\). Based on this DNA string, the VT-based check string \([GCT]\) is also generated. (B) shows the timely detection during the decoding process. (C) illustrates the process of saturated reverse search. Through 13 searches, two candidate strings are obtained. (D) shows the final check process using VT-based check. Only one of the two candidates meets the correct check value and we finally have a unique solution.
number depends on the outgoing arcs of the current working vertex in $D$. If an adjustment results in a string that (partly) corresponds to a valid directed path on $D$, we consider it as a possible correct candidate. This process works if there is only one edit error. In case there are multiple edit errors, as long as the errors are separated enough, we may repair the errors sequentially.

It might happen that the saturated reverse search provides massive candidates, especially when the constraints are not too strict. We further apply a sieving method by tools from coding theory, in order to significantly reduce the number of candidates or even find a unique solution (Figure S4). The trick is to apply an idea similar to the Varshamov-Tenengolts (VT) code [59, 63] and its variations, which play an important role in the current study of error-correcting codes against deletions or insertions. What we do is to provide a “salt-protected” DNA string of length $(\ell + 1)$ as a suffix for each codeword. By salt-protection [50], the suffix is guaranteed to be correct. It stores some information known as the check value $y_{\text{check}}$ for a codeword $y$ (Appendix A3).

The full process of our correction strategy is summarized in Algorithm 3, which is defined as

$$Y_{\text{repair}} = C(y_{\text{wrong}}, y_{\text{check}}, D).$$

(2)

First, we use the saturated reverse search to find a list of candidates. Then for each candidate we compute its check values and see if it matches the correct values stored in the salt-protected suffix. An example of detailed path-based correcting is shown in Figure 3. While it is still possible that the algorithms may fail when facing multiple and dense errors, it behaves well in our simulation (see Evaluation).

**Algorithm 3:** Repair DNA string through path-based correcting

**Input:** wrong DNA string $y_{\text{wrong}}$, path check string $y_{\text{check}}$, and digraph $D$.

**Output:** repaired DNA string set $Y_{\text{repair}}$.

1. Create two empty set $Y_{\text{search}}$ and $Y_{\text{repair}}$.
2. Put $y_{\text{wrong}}$ into an empty candidate set $Y_{\text{candidate}}$.
3. If $Y_{\text{candidate}}$ becomes an empty set, jump to step 9; otherwise, go to step 4.
4. Take out a candidate DNA string $y_{\text{candidate}}$ from $Y_{\text{candidate}}$.
5. If $y_{\text{candidate}}$ belongs to a path in $D$, put $y_{\text{candidate}}$ into $Y_{\text{search}}$ and jump to step 3; otherwise, go to step 6.
6. Obtain $p$ as the position where the path error first occurs in $y_{\text{candidate}}$.
7. Use $D$ to do saturated reverse search for the position $p$ to position $(p - \ell)$ of $y_{\text{candidate}}$.
8. Collect all local repairable DNA strings in step 7 into $Y_{\text{candidate}}$, then jump to step 3.
9. Put DNA strings that their path check string equals $y_{\text{check}}$ from $Y_{\text{search}}$ into $Y_{\text{repair}}$.
10. Output $Y_{\text{repair}}$.

### 3.3 mapping shuffling

Now we consider a variant of SPIDER-WEB which provides a certain amount of additional security against an eavesdropper who has access to $y$ and is curious about $x$.

Once the eavesdropper knows the underlying graph $D$ then trivially he can decode $x$ from $y$. Therefore, the trick is to adjust $D$ as a private key between the source and the receiver. As mentioned in the binding process, the bound information "(nucleotide | digit)" of each outgoing arc is according to a predetermined partial order (Figure 2C). The source and the receiver might pick a random shuffling on the rules for binding the digits and thus make the underlying graph $D$ not completely known to the eavesdropper. For example, as illustrated in (Figure 2C), the source and receiver might agree on the right table as the binding rules. If the eavesdropper uses
the left table for decoding he will be in vain. Thus, by using random shuffling, $D$ becomes private and can serve as a symmetric encryption.

As an example, for a vertex $v$ in $D$, support the order of its outgoing arcs is $C < G < T < A$ and $v$ contains three outgoing arcs (A-arc, G-arc, and T-arc). These arcs will be bound as $\{G|0\}$-arc, $\{T|1\}$-arc, and $\{A|2\}$-arc. In Figure 2C, the label order of different vertices may be different, we consider using a matrix $M$ (called shuffled matrix) to store the such order of each vertex in $D_4^\ell$ (Appendix B1). By this, encoding the binary message $x$ can be expanded as:

$$y_{\text{shuffle}} = E(x, M).$$

Specifically, $M$ changes the finding and setting process in line 6 and line 7 of Algorithm 2.

4 EVALUATION

In this section, we illustrate some benchmark results, to evaluate SPIDER-WEB through different properties, including compatibility, code rate (and stability), repairability, and encryption capability.

4.1 biochemical constraint setup

Since one of our main motivations is to automatically create coding algorithms to deal with arbitrary local biochemical constraints, we need to introduce multiple representative biochemical constraints in the evaluation process. Based on the description in Background, let the observed length $f$ to be 10, we have designed 12 biochemical constraint sets for meeting the actual situations (Table 2), which involve the compatibility of different instruments and different storage approaches (in vivo and in vitro). The order of the constraint set depends on its level of strictness, which is defined as the capacity of their corresponding state-transition digraph (Figure S2).

### Table 2. Investigated biochemical constraint sets

| constraint set | long homopolymer | regionalized GC content | undesired motifs | capacity |
|----------------|------------------|--------------------------|------------------|---------|
| 01             | 2                | 50%                      | restriction sites | 1.0000  |
| 02             | 1                | N/A                      | N/A              | 1.5850  |
| 03             | N/A              | 10% ~ 30%                | N/A              | 1.6302  |
| 04             | 2                | 40% ~ 60%                | similar structures | 1.6698 |
| 05             | 2                | 40% ~ 60%                | N/A              | 1.7761  |
| 06             | N/A              | 50% ~ 70%                | N/A              | 1.7958  |
| 07             | 3                | 40% ~ 60%                | N/A              | 1.8114  |
| 08             | 4                | 40% ~ 60%                | N/A              | 1.8152  |
| 09             | 3                | N/A                      | N/A              | 1.9824  |
| 10             | 4                | N/A                      | N/A              | 1.9957  |
| 11             | 5                | N/A                      | N/A              | 1.9989  |
| 12             | 6                | N/A                      | N/A              | 1.9997  |

Undesired motifs in constraint set “01”: AGCT, GACGC, CAGCAG, GATATC, GGTACC, CTGCAG, GAGCTC, GTCGAC, AGTACT, ACTAGT, GCATGC, AGGCCT, TCTAGA [52];

Undesired motifs in constraint set “04”: AGA, GAG, CTC, TCT [68].

N/A (“not applicable”) represents the constraint set does not contain this type of constraint.

The approximation of capacity of each constraint set is mentioned Appendix A2 and B3.
4.2 comparisons on coding stability

Recently, there are three well-established coding algorithms that can deal with arbitrary local biochemical constraints: DNA Fountain [17], Yin-Yang Code [47], and HEDGES [50]. The influence of biochemical constraints on these three algorithms (and our proposed graph-based encoding) needs to be further investigated.

Here, we designed a simulated code rate experiment for the above-mentioned coding algorithms. In this experiment, 100 groups of the above-mentioned algorithm parameter and 100 bit matrices with size of 72 Kilobytes (including 8 Kilobytes index range and 64 Kilobytes payload range) are randomly introduced to calculate the code rate (nucleotide number / 64 Kilobytes) of 4 investigated coding algorithms.

In Figure 4, the code rate is closely related to the level of strictness of the biochemical constraints. It clearly demonstrates that SPIDER-WEB performs better in encoding tasks than the other three advanced coding algorithms, which can be considered the relatively best and stable selection in the simulation experiment.

Furthermore, the code rate obtained from SPIDER-WEB is close to the corresponding capacity in most cases (Figure S2). In principle, both SPIDER-WEB and HEDGES use modulo operation to extract the nucleotide information of the corresponding position from the binary message. The difference is that SPIDER-WEB performs additional binary operations, which further increases the code rate while reducing a small amount of stability. The average code rate of SPIDER-WEB is 1.09612 times that of HEDGES under 12 constraint sets. Meanwhile, the standard deviation of code rate in SPIDER-WEB increases by 0.00091 on average (than HEDGES).

Figure 4. Evaluation of encoding stability of selected coding algorithms. Red, yellow, green, and purple patches correspond to the encoding results of SPIDER-WEB, HEDGES, Yin-Yang Code and DNA Fountain respectively. Point in each violin plot refers to the median value of data. “×” refers to the coding algorithm unable to obtain DNA string satisfying the constraints within the effective number of iterations.

3 (1) virtual vertex in SPIDER-WEB. (2) mapping between 2 bits and 1 nucleotide in HEDGES [50]. (3) rule index in Yin-Yang Code [47]. (4) two parameters, c and δ, of the Soliton distribution [37] in DNA Fountain, the interval [27] of which are set as c ∈ [0.1, 1] and δ ∈ [0.01, 0.1].
We also find that biochemical constraints, binary patterns, and/or parameter settings have significant effects on the code rate of screen-based coding algorithms (DNA Fountain and Yin-Yang Code). Potentially, a difference between screen-based coding algorithms and others can be attributable to the instability of stochastic processes. In constraint set "09", the difference of DNA Fountain can reach 0.66990, and that of Yin-Yang Code can reach 0.88197. Intriguingly, for some extremely strict biochemical constraints, from constraint set "01" to "06", DNA Fountain cannot generate a valid DNA string within the effective iteration (≤ $10^6$) after more than 10,000 different attempts. It implies a pitfall that the code rate may be much lower than the expectation of users after using this kind of coding algorithm with a specific parameter setting to encode a specific digital file.

4.3 evaluation of path-based correcting

Some early designs [8, 9, 25] were evaluated through experimental case studies rather than large-scale simulated experiments. Besides, as an advanced design, HEDGES [50] did not achieve the adaption with arbitrary biochemical constraints. Thus, it is difficult to compare the previous designs with path-based correcting equivalently.

In this work, we design numerically analysis for the probability of correcting the wrong string (called correction rate $P$ below) to demonstrate the performance of path-based correcting. Specifically, the influences of the length of DNA strings, the number of errors, and the setup of biochemical constraints on the correction rate are investigated. After introducing 12 constraint sets, we considered oligo lengths (100nt ~ 400nt) and long fragment lengths (1000nt ~ 4000nt) for the length setup of DNA strings and the 1 ~ 16 edit errors in DNA strings. A string length, an error number, and a constraint set form an intervention group. In each intervention group, 2,000 randomized trials were conducted with a random seed of 2021.

For each constraint set, the correction rates under different constraint sets are shown in Figure 5A. When there is only one error in the DNA string, SPIDER-WEB has a 100% chance to correct it. This part of the results can tie well with previous proofs of VT code on single error correction [1, 63]. Besides, repaired DNA string set is jointly satisfying validity on established constraints and inspection on path check. With the easing of constraints (from constraint set "01" to "12"), the impact of error rate on correction rate becomes more serious. For an error rate of 2%, the Pearson correlation coefficient between valid number 4 and correction rate is −0.81 (Figure S3A).

Further, Figure 5B reports the correction rates under different string lengths and error rates under constraint set "01". For the repair of multiple errors, the correction rate of SPIDER-WEB will be exponentially reduced with the increase of error number (Pearson correlation coefficient is −0.83, Figure S3B). Meanwhile, under the same number of errors, the shorter the DNA string length, the higher probability of the error aggregation. Patch groups with the same introduced error times (e.g. 4%-100nt, 2%-200nt, and 1%-400nt) in Figure 5B jointly represent an obvious negative impact.

If the repair solution cannot be obtained, such DNA string should be re-obtained which can be regarded as the additional reads. To evaluate the practicability of path-based correcting, we further investigate the minimum reads for correction (or complete repair) and the simulated repair runtime. Considering our proposed correction is probabilistic, the minimum reads (to achieve 100% recovery) could be adjusted as $1 + \sum_{t=1}^{\infty} (1 - P)^t = 1/P$, where $P$ is the correction rate in the current situation. Intriguingly, Figure 5C illustrates the minimum reads for correction linearly decrease with the increase of DNA string length under the same number of errors. When 3 edit errors occur in the DNA string of length 100, the minimum reads could be 4 (specifically 3.06). This indicates that the full recovery can only be guaranteed when the minimal sequencing coverage reaches 4x. As another common parameter, results indicated that the average repair runtime is less than 1 second (Figure 5D). Practically, for multiple error repair, we also design the time-consuming analysis for long fragments (1000nt ~ 4000nt) to provide references for in vivo storage. Although the looser the constraints, the more the number of saturated reverse

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4 The valid number refers to vertex set size of digraph screened from the constraint set.
SPIDER-WEB is all you need

Figure 5. **Evaluation of probabilistic correction by path-based correcting.** (A) represents the influence of constraint sets and error rates on correction rates. The length of DNA string in the panel is set as 100nt. (B) illustrates the correction rates under different lengths and different error rates. The constraint set in this panel is set as "01". (C) reports the minimum read(s) for correction (or complete repair) in different DNA lengths or different error numbers under constraint set "01". (D) calculates the average runtime of path-based correcting in different DNA lengths or different error numbers under constraint set "01" (using Intel i7-4710MQ @ 2.50GHz).

As a function of storage management, data protection and security is necessary. It implies that the difficulty of deciphering DNA molecules and obtaining binary messages during transmission should be further considered.

Based on graph-based encoding, DNA molecules maintain plaintext form. As mentioned in Methodology, when the eavesdropper knows the underlying graph $\mathcal{D}$ then he can obtain the original information from DNA molecules. In order to simulate the actual eavesdropping difficulty, we randomly generate DNA strings from $\mathcal{D}$ until the obtained set of generated strings contains all the vertices in $\mathcal{D}$. When all vertices are accessed, the searches will increase, the detection probability will also decrease significantly (Figure 5A). The average runtimes for 12 constraint sets show that the correction process of a DNA string usually does not take more than 1 minute.
obtained vertices can be connected according to the de Bruijn mechanism (see Methodology), then \( D \) is created. Once \( D \) is created then the eavesdropper can obtain stored information.

Through 100 randomized experiments, the difficulty of restoring \( D \) from random DNA strings is shown in Figure 6A. Meanwhile, Figure S5 further demonstrates the decoding difficulty increases exponentially with the increase of the number of vertices in the digraph. For a digital file with unbiased byte frequency distribution, even the digraph with the most vertices in the simulation experiment (constraint set “12”) can be restored with less than the size of 234MB. Although errors in DNA molecules can increase such difficulty, we conservatively believe that graph-based encoding is in the deciphering crisis.

![Figure 6](image_url)

**Figure 6.** Results related to encryption. (A) represents the size range of the digital file required to reconstruct the coding graph. In this experiment, we set the length of string as 100nt and the random seed as 2021. The number of DNA strings is converted into transmitted data capacity through the capacity approximation (Appendix A2). (B) represents the relationship between DNA string length and bits of security in coding graphs obtained under different biochemical constraints. Through the mapping shuffling in SPIDER-WEB, the original nucleotide-digit mapping of some or all vertices of \( D \) will be disrupted. By averaging, estimation of bits of security [5] can be

\[
\sum_{i=1}^{n} \log_2(i) \times \frac{|V_i|}{|V|} + \log_2(6) \times |V_3| + \log_2(24) \times |V_4|
\]

where \( V_i = \{v | v \in V, \deg_D(v) = i\} \) and \( n \) is the payload length in DNA string. This equation averages the information entropy of each vertex: for a vertex with the out-degree \( i \), the nucleotide-digit mapping has \( i! \) choices after mapping shuffling, the corresponding entropy of which could be \( \log_2(i!) \). Assume the probability of each vertex being accessed is equivalent, take average according to the out-degree frequency of \( V \), and the bits of security can be estimated. Therefore, the equivalent key strength under different constraint sets is reported in Figure 6B. Obviously, the DNA string generated by most coding graphs (constraint set “02” ~ “12”) can achieve the ideal key strength [28] when its length is no longer than 100nt. Such length of DNA string is the payload length that can be easily reached in the form of in vitro storage [17, 25, 47, 50].

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**Methodology**

1. **Randomized Experiment:**
   - Set the length of string as 100nt and the random seed as 2021.
   - Convert the number of DNA strings into transmitted data capacity.
   - Measure the decoding difficulty.

2. **Graph Construction:**
   - Use the de Bruijn mechanism to connect vertices.
   - Create the digraph \( D \).

3. **Bits of Security Estimation:**
   - Calculate the information entropy using the formula:
     \[
     \sum_{i=1}^{n} \log_2(i) \times \frac{|V_i|}{|V|} + \log_2(6) \times |V_3| + \log_2(24) \times |V_4|
     \]
   - Average the entropy according to the out-degree frequency.

4. **Key Strength:**
   - Report the equivalent key strength under different constraint sets.
   - The ideal key strength is achieved when the DNA string length is no longer than 100nt.

---

**Figure 6A:**
- **Transmitted File Size vs. DNA String Length:**
  - Data points for various constraint sets (KB, MB, GB, TB).
- **Bits of Security:**
  - Data points for different DNA string lengths.

**Figure 6B:**
- **DNA String Length vs. Bits of Security:**
  - Graphs for constraint sets [01, 02, 03, ..., 11, 12].
  - AES-256 represented by a line.

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**Conclusion:**
- Graph-based encoding is in the deciphering crisis.
- The ideal key strength is achieved under specific conditions.
- DNA string lengths that can be easily reached in vitro storage are reported.
5 CONCLUSION

Recently, biochemical constraints and their combinations are becoming more diverse. In-depth investigation on them to be compatible with abundant storage requirements also becomes more necessary. Therefore, SPIDER-WEB is to establish the graph-based coding algorithms under the above constraints automatically. From the aspect of the application, SPIDER-WEB can be used directly by setting attributes of local biochemical constraints in the program. With a broader view, echoing capacity approximation (Appendix A2 and B3), graph-based coding algorithms generated by SPIDER-WEB could provide a benchmark for the follow-up coding algorithm design of DNA-based storage.

Considering the errors of DNA molecules introduced in synthesis, sequencing and other biochemical operations, we constructed path-based correcting in SPIDER-WEB to correct substitution, insertion, and deletion errors in a probabilistic way. For information stored in oligo pools (100nt ~ 400nt), within the 1.34% end-to-end error rate [50], SPIDER-WEB can repair 48.1% of wrong DNA sequences (sequencing coverage is 1). With the help of expectation, suggesting a coverage of 4 is sufficient to achieve an error-free information retrieval. Although the our proposed search in the correcting process might have a potential high time complexity, the recovery runtime is acceptable at less than 1 second for each sequence (≤4 errors and ≤400nt length). For longer DNA sequences or more errors, path-based correcting will require a higher sequencing coverage to support its repair capabilities.

As an additional option, mapping shuffling in SPIDER-WEB provides necessary encryption for cold data, which can be compatible with the existing range of payload length.

In summary, SPIDER-WEB develops a set of fundamental tools, for the existing and potential challenges of DNA-based storage, including compatibility, repairability, encoding stability and encryption capability.

6 FUTURE WORKS

SPIDER-WEB can be a fundamental tool for DNA-based storage algorithm development. Based on this work, some issues could be further investigated.

(1) Arbitrary local constraints need to be expanded into arbitrary global constraints. By doing so, we should have the opportunity to include the minimum free energy [47], toxic DNA strings [29, 54], or long continuous regions of spurious hybridization for DNA strand displacement [72] into consideration.

(2) The existing mathematical models for biochemical constraints are mainly regarded as simply valid/invalid classification. Regression models may be further designed for quantitatively describing the compatibility of encoded DNA strings with techniques or storage environments, which will help to explore a more accurate optimal trade-off between biochemical constraints and code rate.

(3) Considering the issue of code rate stability, the graph-based coding algorithm with fixed-length could be further designed at the expense of a certain code rate.

(4) It is worth investigating how to integrate some more advanced error correction codes into the path-based correcting module.

CODE AVAILABILITY

Kernel codes of SPIDER-WEB are exhibited in the “dsw” folder of the GitHub repository [73]. This repository also includes the process codes of all simulation experiments in its “experiments” folder. The usage examples of each method or class and customized suggestions are further described on the ReadtheDocs website [74].

DATA AVAILABILITY

The link of simulated data underlying this article is shared on the above-mentioned GitHub repository.
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AUTHOR CONTRIBUTIONS

H.Z, W.Z, X.X, Z.P, and Y.S designed local biochemical constraint sets; H.Z designed the graph-based encoding; H.Z and Z.L designed the saturated reverse search and Varshamov-Tenengolts-based path check; H.Z designed the mapping shuffling; H.Z implemented Python codes and Y.Z evaluated them by MATLAB codes; Z.L and Y.Z constructed the capacity approximator and completed the mathematical proofs; H.Z prepared the figures and tables; H.Z and Z.L mainly drafted the manuscript; Y.Z, Z.P, Y.S, W.Z, and X.X revised the manuscript; Y.S and Y.Z supervised the study jointly. All authors read and approved the final manuscript.

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Supplementary Materials

A  MATHEMATICAL REPRESENTATIONS AND PROOFS

A.1 mathematical formulation of the local biochemical constraints

According to the previous works [8, 14, 17, 22, 24, 25], two kinds of biochemical constraints are widely investigated: the homopolymer run-length constraint and the regionalized GC-content constraint.

In a DNA string, a homopolymer run refers to a maximal consecutive sub-string of the same symbol and the number of nucleotides in each run is called its run-length. For example, $v = \text{AAAAATTCGG}$ contains four runs with run-lengths as 4,2,1,2 accordingly. Typically in DNA-based storage long runs should be avoided. A homopolymer run-length constraint is of the form “the maximal run-length is at most $h$”.

Another constraint, known as the regionalized GC-content, requires that the GC-ratio in any consecutive sub-string of length $\ell$ is bounded within an interval. The parameter $\ell$ is called the observed length and usually $\ell \geq h$. Given $0 \leq \epsilon_1 \leq \epsilon_2 \leq 1$ and the observed length $\ell$, a regionalized GC-content constraint is of the form “in any consecutive sub-string of length $\ell$, the sum of the numbers of G and C is within the interval $[\epsilon_1 \times \ell, \epsilon_2 \times \ell]$.”

Additionally, we sometimes come across a third kind of constraint regarding undesired motifs. Such motifs usually have a serious impact on specific biochemical operations or storage environments. Let $\Theta$ be a set of undesired motifs. A DNA string $v$ is $\Theta$-free if each motif in $\Theta$ does not appear in $v$ as a consecutive sub-string. Usually we only consider undesired motifs with length less than or equal to the observed length $\ell$.

A.2 capacity approximation for coding algorithms under specific biochemical constraints

Given a set of constraints $C$, we want to encode binary messages into quaternary DNA strings satisfying the constraints. In classical coding theory, we usually want the encoded codewords to be of a fixed length. That is, we want to encode binary messages from $\{0, 1\}^k$ into quaternary DNA strings in $\{A,C,G,T\}^n$. The efficiency of the code is characterized by the code rate $r$, defined as $r = k / n$. The maximal code rate is called the capacity of such codes. For example, if there are no constraints, then by a trivial mapping from $\{00, 01, 10, 11\}$ to $\{A,C,G,T\}$, we have a code of rate 2.

However, due to the constraints $C$ we must have a sacrifice on the code rate. Let $\Sigma_C^n$ be the set of DNA strings in $\{A,C,G,T\}^n$ which satisfy the constraints $C$. For any $k$, the maximal code rate will be $k / n'$ where $n'$ is the least integer such that $|\Sigma_C^n| \geq 2^k$. The precise computation of $|\Sigma_C^n|$ is a difficult problem. In constrained coding theory, a standard way to asymptotically compute $|\Sigma_C^n|$ relies on the celebrated Perron-Frobenius Theorem [21, 45] and the procedure is as follows. Given the constraints $C$, consider its state-transition digraph $D_C^\ell$, where $\ell$ is chosen as the observed length in a regionalized GC-content constraint from $C$. In fact, such a graph is exactly the graph we mentioned in Algorithm 1 after the screening process (without trimming). As long as $D_C^\ell$ is strongly-connected, the spectral radius $\rho$ of the adjacency matrix of $D_C^\ell$ will provide the estimation

$$\lim_{n \to \infty} |\Sigma_C^n| \approx \rho^n,$$

and thus the capacity is upper bounded by $\log_2 \rho$.

While coding theorists might focus on fixed-length coding algorithms with rate approaching $\log_2 \rho$, in this paper we consider variable-length codes. That is, our SPIDER-WEB coding algorithm might encode binary messages from $\{0, 1\}^k$ into DNA strings of variable lengths. For a comparison with the fixed-length model, for any variable-length coding algorithm $E$, define $\bar{n} = \frac{1}{k} \sum_{x \in \{0,1\}^k} |E(x)|$ to be the average length of the encoded codewords and the rate of $E$ is defined as

$$r(E) = k / \bar{n}.$$ 

Since fixed-length codes are special cases of variable-length codes, at first sight we might expect $r(E)$ to be larger than the capacity of fixed-length codes. However, a key observation is that $\log_2 \rho$ is still an upper bound
of \( r(E) \). Following this observation, we may compare the code rate of SPIDER-WEB and the corresponding capacity result and it gives supportive evidences that our SPIDER-WEB coding algorithms indeed have good code rates. The rest of this subsection is devoted to the proof of the key observation:

\[
\lim_{k \to +\infty} r(E) \leq \log_2 \rho, \tag{5}
\]

**Proof of Equation 5.** Let the set of binary messages be \( \{0, 1\}^k \) where \( k \) can be arbitrarily large. Let \( n' \) be the smallest integer such that \( |\Sigma'_{c_i}| \geq 2^k \). A fixed-length code will have rate \( k / n' \). A variable length code, however, could use codewords with smaller length. Without loss of generality, we assume in a greedy way that this variable length code uses codewords with length as small as possible. Divide all the \( 2^k \) codewords into sets \( \mathcal{E}_1, \mathcal{E}_2, \ldots, \mathcal{E}_{n'} \) where \( \mathcal{E}_i \) represents the set of codewords of length \( i \). Then by definition \( \pi = \frac{1}{2^k} \sum_{i=1}^{n'} i |\mathcal{E}_i| \).

Pick an auxiliary variable \( 0 < \epsilon < 1 \), divide \( \sum_{i=1}^{n'} i |\mathcal{E}_i| \) into two parts and the computation proceeds as follows.

\[
\frac{1}{r(E)} = \frac{1}{k \times 2^k} \sum_{i=1}^{n'} i |\mathcal{E}_i| = \frac{1}{k \times 2^k} \times \left( \sum_{i=1}^{\epsilon \times k} i |\mathcal{E}_i| + \sum_{i=\epsilon \times k + 1}^{2^k} i |\mathcal{E}_i| \right) \geq \frac{1}{k \times 2^k} \times \left( \sum_{i=1}^{\epsilon \times k} i |\mathcal{E}_i| + \frac{\epsilon \times k}{\log_2 \rho} \times \sum_{i=\epsilon \times k + 1}^{2^k} \log_2 |\mathcal{E}_i| \right). \]

By substituting \( \sum_{i=1}^{\epsilon \times k - 1} |\mathcal{E}_i| = 2^k - \sum_{i=\epsilon \times k}^{2^k} |\mathcal{E}_i| \), we arrive at

\[
\frac{1}{r(E)} \geq \frac{1}{k \times 2^k} \times \sum_{i=1}^{\epsilon \times k} i |\mathcal{E}_i| + \frac{\epsilon}{2^k \times \log_2 \rho} \times \left( 2^k - \sum_{i=1}^{\epsilon \times k} |\mathcal{E}_i| \right) = \frac{\epsilon}{2^k \times \log_2 \rho} + \frac{1}{2^k} \times \sum_{i=1}^{\epsilon \times k} \left( \frac{i}{k} - \frac{\epsilon}{\log_2 \rho} \right) |\mathcal{E}_i|.
\]

Note that for \( 1 \leq i \leq \epsilon \times k \), we have \( \frac{i}{k} - \frac{\epsilon}{\log_2 \rho} \leq 0 \). According to Equation (4), \( |\mathcal{E}_i| \leq |\Sigma'_{c_i}| = \rho^i \). Then we proceed as follows.

\[
\frac{1}{r(E)} \geq \frac{\epsilon}{2^k \times \log_2 \rho} + \frac{\epsilon \times k}{2^k \times \log_2 \rho} \times \left( \frac{1}{k} - \frac{\epsilon}{\log_2 \rho} \right) |\mathcal{E}_i| \geq \frac{\epsilon}{2^k \times \log_2 \rho} + \frac{1}{2^k} \times \sum_{i=1}^{\epsilon \times k} \left( \frac{i}{k} - \frac{\epsilon}{\log_2 \rho} \right) \rho^i.
\]

Denote \( \Delta = \sum_{i=1}^{\epsilon \times k} \left( \frac{i}{k} - \frac{\epsilon}{\log_2 \rho} \right) \rho^i \). By calculating the difference between \( \rho \times \Delta \) and \( \Delta \) we have

\[
(\rho - 1) \times \Delta = -\frac{1}{k} \times \sum_{i=1}^{\epsilon \times k} \rho^i + \frac{\epsilon \times k \rho \times \log_2 \rho}{\log_2 \rho} \geq -\frac{1}{k} \times \rho^{\epsilon \times k} \times (\rho - 1) = \frac{1}{k} \times (\rho - 1) \leq \frac{\rho}{k \times (\rho - 1)}.
\]

Then finally we have

\[
\frac{1}{r(E)} \geq \frac{\epsilon}{2^k \times \log_2 \rho} + \frac{\rho \times 2^{\epsilon \times k}}{2^k \times k \times (\rho - 1)^2} + \frac{\rho}{2^k \times k \times (\rho - 1)^2}.
\]

When taking \( k \to +\infty \), the second and the third term on the right hand side approach zero and thus we have proven \( \lim_{k \to +\infty} \frac{1}{r(E)} \geq \frac{\epsilon}{\log_2 \rho} \). Since the parameter \( \epsilon \) can be chosen arbitrarily close to 1, we have finally proven \( \lim_{k \to +\infty} r(E) \leq \log_2 \rho \). \( \square \)
A.3 modular operation setting of the “salt-protected” suffix storing check values

Consider an arbitrary map between \( \{A, C, G, T\} \) and \( \{0, 1, 2, 3\} \), say \( A \leftrightarrow 0, C \leftrightarrow 1, G \leftrightarrow 2, \) and \( T \leftrightarrow 3 \).

For a quaternary DNA string \( y = (y[1], y[2], \cdots, y[n]) \), define its signature as \( \text{sig}(y) = (x[1], x[2], \cdots, x[n - 1]) \), where \( x[i] = 1 \) if \( y[i + 1] \geq y[i] \) and \( x[i] = 0 \) if \( y[i + 1] < y[i] \).

The check value \( y_{\text{check}} \) for a codeword \( y \) is of length \( \ell + 1 \) and consists of two parts. The first entry of \( y_{\text{check}} \) is the value \( \sum_{i=1}^{n} y[i] \pmod{4} \). The last \( \ell \) entries is the quaternary expression of the number \( \sum_{i=1}^{n-1} i \times x[i] \pmod{4^\ell} \).

The idea behind these check values is the celebrated Varshamov-Tenengolts code \([59, 63]\). If there is only one deletion or one insertion, then from the check values we may precisely correct the error. In this paper we do not directly apply the decoding algorithms of VT codes or their variations, since they are vulnerable against multiple errors. Instead, we only use these check values as a sieving method, to further check the correctness for each candidate string arisen from the saturated reverse search method.

B IMPLEMENTATIONS AND OPTIMIZATIONS

For graph-related calculations, the adjacency matrix is conventionally used to represent a digraph. For the observed length \( \ell \) mentioned above, the shape of an adjacency matrix is \( 4^\ell \times 4^\ell \). When \( \ell \) reaches 8, the file size will achieve 16.0 Gigabytes with integer format \([75]\), which is unable to be allocated (both MATLAB and Python platforms). Considering that such adjacency matrix is an extremely sparse matrix, only 4 positions in each row \( (4^\ell) \) can be actually used. Using the compressed matrix such as compressed sparse row can save memory space exponentially, but it brings trouble to massive matrix operation (e.g. singular value decomposition). It implies that the computing time of capacity approximation, coding algorithm generation, and graph-based search may be intolerable. Thus, in this work, we have completed some design and optimization at the software level to make the creation and calculation of huge digraphs possible.

B.1 representation of digraph

Let \( A = 0, C = 1, G = 2, \) and \( T = 3 \), the index of a vertex in \( \mathcal{V}_4^\ell \) can be defined as a decimal number:

\[
\text{index}(u) = \sum_{p=1}^{\ell} u[p] \times 4^{\ell-p}.
\]

where \( u \) is \( i \)-th vertex of \( \mathcal{V}_4^\ell \). Such index belongs to \( [0, 4^\ell - 1] \cap \mathbb{N} \).

Assuming \( u \in \mathcal{V}_4^\ell \) and \( \mathcal{V} \subseteq \mathcal{V}_4^\ell \), we first consider using a vertex vector \( \gamma \) with the length of \( 4^\ell \) to describe the relationship between \( u \) and \( \mathcal{V} \): if \( \gamma[\text{index}(u)] = 1, u \in \mathcal{V} \); otherwise, \( u \notin \mathcal{V} \). The vertex vector of \( \mathcal{V}_4^\ell \) is an all-one vector. For a sub-graph \( \mathcal{D} = (\mathcal{V}, \mathcal{A}) \) of \( \mathcal{D}_4^\ell \), we can easily conclude that \( i \) and \( j \) must satisfy the below three conditions if \((\mathcal{V}_4^\ell[i], \mathcal{V}_4^\ell[j])\) is an arc of \( \mathcal{A} \): \( \gamma[i] = 1, \gamma[j] = 1, \) and \( i \mod 4^{\ell-2} = j/4 \).

In order to remove the space occupied by a large amount of useless information in the above-mentioned adjacency matrix of \( \mathcal{D} \), we further construct a special compressed matrix, named accessor \( \alpha \). \( \alpha \) contains \( 4^\ell \) rows (for vertex indices) and 4 columns (for outgoing arcs), if \( (\mathcal{V}_4^\ell[i], \mathcal{V}_4^\ell[j]) \) is an arc of \( \mathcal{A} \), \( \alpha[i, j \mod 4] = j \). The value in remaining cells of \( \alpha \) will be set as \(-1\).

The value of each cell in accessor is carefully designed. Since the encoding/decoding process and other graph-based operations (capacity approximation and path search) involve a large number of vertex access and/or jump operations, each cell of accessor is set as the index information of its follow-up vertex, that is, quick access to \texttt{key} through value. Through this way, these operations do not require additional calculations. Besides, location “\(-1\)“ in Numpy package refers to the last location in vector. For example, we have a vector \( \gamma = (1, 2, 3, \cdots, 10)^{10 \times 10}, \gamma[-1] = \gamma[10] = 10 \). In the implementation, we only need to allocate a vector of size \( 4^\ell + 1 \), so that the operation of these irrelevant values (for “\(-1\)“ location) takes place in the last position. By removing the last position after calculations, the expected vector will be obtained.
To illustrate the difference between adjacency matrix and accessor, we represent the digraph in Figure 3A by the following two kinds of matrices 5:

\[
\begin{bmatrix}
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\end{bmatrix}
\Rightarrow
\begin{bmatrix}
-1 & -1 & -1 & -1 \\
4 & -1 & -1 & 7 \\
8 & -1 & -1 & 11 \\
-1 & 1 & 2 & -1 \\
-1 & -1 & -1 & -1 \\
-1 & -1 & -1 & -1 \\
-1 & 13 & 14 & -1 \\
-1 & 1 & 2 & -1 \\
-1 & -1 & -1 & -1 \\
-1 & -1 & -1 & -1 \\
4 & -1 & -1 & 7 \\
8 & -1 & -1 & 11 \\
-1 & -1 & -1 & -1 \\
\end{bmatrix}
\]

By doing so, the memory usage of digraph in this work can be reduced from \(4^\ell\) to \(4^{\ell+1}\). In our simulation experiments (\(\ell = 10\)), the allocated memory is decreased from 4.0 Terabytes to 16.0 Megabytes (262,144 times).

B.2 use of rapid search in digraph

In the generation task of SPIDER-WEB, breadth first search [39] is used widely times (line 2 ~ 5 of Algorithm 1). Meanwhile, in the repair task of SPIDER-WEB, a major repetitive operation (line 5 ~ 8 of Algorithm 3) is to detect whether the repaired DNA string is on the path that satisfies established biochemical constraints. Thus, the optimization of search operation in digraph can significantly reduce the time required for not only graph generation but also path-based correction.

Through accessor, the search operation can be converted to matrix operation, we therefore construct a rapid search for layers and paths. Here, we introduce stride \(s\) to represent the stride from the root vertex to the leaf vertex, then provide below customized algorithms. Normally, in the breadth first search, the vertex acquisition of the next layer depends on the next vertex of each vertex in the current layer. Accessor considers parallelize the search of each layer, so as to speed up the search. Taking \(i\)-th vertex \((\mathcal{V}_i[r])\) as the root vertex in the digraph \(\mathcal{D}\), the initial vector can be \(y_0 = (0, 0, \cdots, 1, \cdots, 0, 0)_{1 \times 4^\ell}\). In this vector, only \(i\)-th element is 1. As the next layer, \(y_i\) can be represent as \((0, 0, \cdots, a[i, j] \geq 0, \cdots, 0, 0)_{1 \times 4^\ell}\). where \(j \in \{1, 2, 3, 4\}\) The “where” function (in Numpy package) can calculate this conditional function in parallel. Set \(y_i\) as an all-zero vector, then

\[
y_i[\text{where } a[i] \geq 0] = 1.
\]

Expansively, the \(s\)-layer vector can be defined through \(s\) iterations. From \(i\)-th iteration to \((i + 1)\)-th iteration,

\[
y_{i+1}[\text{where } a[\text{where } y_i \geq 0] \geq 0] = 1.
\]

For the path search, it can be simplified to the connectivity of the root vertex \(\mathcal{V}_0[r]\) and the leaf vertex \(\mathcal{V}_0[l]\) with \(s\) level. Initializing the all-zero vector \(y_0\) with only \(r\)-th element is 1. Through above-mentioned search, if \(y_s[l] = 1\), \(\mathcal{V}_s[r]\) and \(\mathcal{V}_s[l]\) are connected.

5 In the implementation, vector/matrix indices start at 0.
B.3 approximation of largest eigenvalue
As mentioned in Appendix A2, the capacity under the considered biochemical constraints can be approximated based on the largest eigenvalue of its corresponding generated digraph (see Methodology). Theoretically, the largest eigenvalue ($\rho$) can be calculated directly. However, personal laptops cannot carry a complete adjacency matrix from huge graphs. For example, in the Python environment, adjacency matrices larger than $4^8 \times 4^8$ are not allowed to be created by Numpy [62]. This may not be enough for biochemical constraints. Therefore, it is a reasonable choice to calculate $\rho$ with the help of accessor $\alpha$.

Considering that accessor is not good at doing matrix decomposition operations, QR-based method [20] should be replaced by power iteration [38]. Hence, the largest eigenvalue can be approximated as:

$$\rho = \lim_{t \to \infty} \max(y_t),$$

where $y_t$ is the eigenvector obtained from $t$-th iteration and $t$ approaches infinity. $y_t$ is calculated by $y_{t-1}$ and $\alpha$ (see Algorithm 4) and $y_0$ is the all-one vector or a random vector [31]. After infinity iterations, the maximum value of the final eigenvector can be regarded as the largest eigenvalue. In practice, we cannot obtain the result when the iteration approaches infinity. When $\frac{|\max(y_t)| - \max(y_{t-1})|}{\max(y_{t-1})} \leq 10^{-10}$ [19], we believe that there is no difference between the $i$-th largest eigenvalue and $\infty$-th largest eigenvalue.

**Algorithm 4:** Approximate the capacity

| Input: | accessor $\alpha$. |
|---|---|
| Output: | approximated capacity $\rho$. |
1. Set $i$ to be 0 and set the initial eigenvector $y_0$ as the all-one vector $(1, 1, \cdots, 1)_{1 \times 4^2}$.
2. Set $j$ to be 1 and set $y_{i+1}$ as the all-zero vector $(0, 0, \cdots, 0, 0)_{1 \times 4^2}$.
3. Let $y_k$ as the key vector contains indices of the its value in $\alpha^T[j]$ greater than 0.
4. Let $y_v$ as the value vector contains values for location $y_k$ in $y_i$.
5. Set $y_{i+1}[y_k]$ as $y_{i+1}[y_k] + y_v$ and set $j$ as $j + 1$.
6. If $j$ is 4, go to step 7; otherwise, go to step 3.
7. If $\frac{|\max(y_{i+1})| - \max(y_{i})|}{\max(y_{i})} \leq 10^{-10}$, go to step 8; otherwise, set $i$ as $i + 1$ and go to step 2.
8. Output $\log_2(\max(y_{i+1}))$.

B.4 reliability analysis of capacity approximation
Since power iteration is more susceptible to small perturbations of polynomial coefficients [69] than QR-based method, we consider to verify the reliability of our proposed capacity approximation through some experiments.

First of all, we prove that the capacity obtained by our approximation method is consistent with some well-known graphs based on standard definition. Here, we define 4 graphs of length 2:

- $\log_2(1)$: a directed cycle, containing “AC”, “CG”, “GT”, and “TA”.
- $\log_2(2)$: a GC-balanced digraph, screening “AA”, “AT”, “CC”, “CG”, “GC”, “GG”, “TA”, and “TT”.
- $\log_2(3)$: a digraph without homopolymer, screening “AA”, “CC”, “GG”, and “TT”.
- $\log_2(4)$: a complete digraph without screening (4-ary de Bruijn graph of order 2).

As the initial results, there are no difference between results approximated by our proposed method and those by oral arithmetic. And the capacity can be obtained with at most 2 iterations, in other words, the correct result is calculated in the first iteration.
Afterwards, on the basis of the above-mentioned complete graph, we investigate 100 pruned (at random) digraphs for verifying the reliability of our method. In the automated testing, referential capacities can be approximated by Numpy “linalg.eig” function [3, 62]. The difference between these referential capacities and corresponding approximated capacities can also be used to evaluate the latter’s reliability directly. As shown in Figure S6A and B, the random experimental result consists of 100 measurements. It is obvious that the 50% relative error result is between $1.30 \times 10^{-11}$ and $8.16 \times 10^{-11}$. Besides, the median value of relative error is $2.97 \times 10^{-11}$. An obvious trend is that the greater the potential information density of a directed graph, the smaller the relative error (Pearson $-0.67$). For the approximated capacity greater than or equal to 1, the range of relative error is $(2.54 \times 10^{-13}, 2.02 \times 10^{-11})$, which is less than the error tolerance ($10^{-10}$).

Finally, on the basis of the above experiment, we investigate the influence of matrix size change on the relative error. Here, the only adjustable parameter is that the size of investigated adjacency matrices is gradually increased from $4^2 \times 4^2$ to $4^6 \times 4^6$. As shown in Figure S6C, no matter how the observed length changes, the median error remains around $3.05 \times 10^{-11}$. Thus, there is no clear relationship between observed lengths and errors. In addition, since each sub-experiment only completed the capacity error comparison of 100 random digraphs, the results cannot be taken as evidence that increasing the observed length can reduce the sudden high error.

Through these experiments, we believe the relative error of capacity approximation is on the order of minus ten of ten, which is equivalent to error tolerance in the preset values.

C DETAILED INFORMATION

- Figure S1: impact of retaining vertex with out-degree of 1 on code rate. It explains the reason why vertices with a out-degree of 1 is removed when building an underlying digraph in Methodology section.
- Figure S2: code rate of graph-based coding algorithms versus their corresponding approximated capacity. It echoes the performance of SPIDER-WEB in Evaluation section.
- Figure S3: correlation between observed variables in DNA string repair. It provides the basis for the correlation coefficient of probabilistic error correction in Evaluation section.
- Figure S4: effect of Varshamov-Tenengolts-based path check. It reveals that Varshamov-Tenengolts-based path check can find the only repair solution or significantly reduce the number of candidate solutions (see Methodology).
- Figure S5: reason for considering privacy protection capacity. It illustrates the growth trend of transmitted file size in the process of reconstructing the target digraph.
- Figure S6: relative error statistics for random digraphs. It reports the reliability of capacity approximation for Appendix B.
Figure S1. **Impact of retaining vertex with out degree of 1 on code rate.** As the experimental setup, the length of bit is 100, the task repeat time is 100, and the task random seed is 2021. In this experiment, the influence of payload length (or bit length) on code rate is only considered, which does not contain index range, error-correction range, and primer range.
Figure S2. **Code rate of graph-based coding algorithms versus their corresponding approximated capacity.** As the experimental setup, the length of bit is 100, the task repeat time is 100, and the task random seed is 2021. In this experiment, the influence of payload length (or bit length) on code rate is only considered, which does not include index range, error-correction range, and primer range.
Figure. S3. **Correlation between observed variables in DNA string repair.** Here, valid number of each constraint set refers to the vertex set size of its corresponding digraph. (A) shows the correlation between valid numbers and correction rates in 100nt/2%. (B) shows the correlation between introduced error numbers and correction rates.
Figure S4. Effect of Varshamov-Tenengolts-based path check. Here, the word “search-only” refers to using saturated reverse search alone, and the word “combined” represents that the candidates are the solution intersection of the saturated reverse search and the path check. The data is the sub-data (100nt/1%) of Figure 5.
Figure S5. Relationship between graph reconstruction percentage and transmitted file size. Each line is the median value of results, which consists of 100 random tasks.
Figure S6. **Relative error statistics for random digraphs.** In (A) and (B), 100 random directed graphs are pruned from the graph without constraints. Among them, the median value is $2.97 \times 10^{-11}$, the interquartile range is $(1.30 \times 10^{-11}, 8.16 \times 10^{-11})$, the value of outliers is less than $8.26 \times 10^{-13}$ or more than $1.28 \times 10^{-9}$. For (C), 100 random directed graphs of different investigated observed lengths are pruned from the graph without constraints. The median values of each sub-statistics are $2.97 \times 10^{-11}$, $3.37 \times 10^{-11}$, $3.70 \times 10^{-11}$, $2.92 \times 10^{-11}$, and $2.33 \times 10^{-11}$, respectively.