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Preservation of DNA for data storage

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The preservation of DNA has attracted significant interest of scientists in diverse research fields from ancient biological remains to the information field. In light of the different DNA safekeeping requirements (*e.g.*, storage time, storage conditions) in these disparate fields, scientists have proposed distinct methods to maintain the DNA integrity. Specifically, DNA data storage is an emerging research, which means that the binary digital information is converted to the sequences of nucleotides leading to dense and durable data storage in the form of synthesized DNA. The intact preservation of DNA plays a significant role because it is closely related to data integrity. This review discusses DNA preservation methods, aiming to confirm an appropriate one for synthetic oligonucleotides in DNA data storage. First, we analyze the impact factors of the DNA long-term storage, including the intrinsic stability of DNA, environmental factors, and storage methods. Then, the benefits and disadvantages of diverse conservation approaches (*e.g.*, encapsulation-free, chemical encapsulation) are discussed. Finally, we provide advice for storing non-genetic information in DNA *in vitro*. We expect these preservation suggestions to promote further research that may extend the DNA storage time.
The bibliography includes 99 references.

Contents

1. Introduction 280
2. Influence factors of DNA degradation 282
2.1. Intrinsic stability of DNA 282
2.1.1. DNA duplex stability 282
2.1.2. Single-strand DNA stability 282
2.2. Environmental factors 282
2.2.1. Temperature 282
2.2.2. Water 283
2.2.3. UV irradiation 283
2.2.4. Oxidation 283
2.2.5. pH Values 284
2.3. Storage methods 284
3. Evaluation of different preservation methods 284
3.1. Encapsulation-free methods 284
3.1.1. Solution preservation 284
3.1.2. Dehydration 285
3.1.3. Freeze – drying 285
3.1.4. Addition of additives 285
3.2. Chemical encapsulation methods 287
3.2.1. Covalent attachment 287
3.2.2. Non-covalent attachment 288
3.2.3. Layer-by-layer self-assembly 288
3.2.4. Other aspects of preservation 288
4. Conclusion and outlook 289
Abbreviations 289

1. Introduction

In 1869, scientists first discovered that DNA is the carrier of genetic information. Then, numerous DNA secrets have been gradually covered since the genetic code breakthrough in 1960.[1] DNA technology has been applied to many aspects, such as forensics,[2] drug delivery,[3] and digital data storage.[4] It is well-known that organisms depend on nucleic acids to transfer genetic information to future generations for at least several billion years. From the research point of view, the analysis of nucleic acids is essential for further studying DNA. Thus, it is important to keep the maintenance of chemical and physical integrity of DNA molecules, especially intact sequence information.

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Although the preservation of DNA exists in various research fields, from ancient biological remains to DNA barcoding, the requirements on storage time and storage conditions differ significantly. For instance, bioarchaeologists should deal with some nuclear DNA in ancient permafrost samples preserved for a million years.[5] Biologists only need to successfully preserve filtered macrobial environmental DNA at room temperature in buffer solution over two weeks.[6] In contrast, the preservation of DNA in the data storage means that the binary digital information is converted to the sequences of nucleotides, contributing to dense and durable data storage in the form of synthesized DNA. The synthesized DNA will then be in a state of protection through physical or chemical treatment for long-term storage.[7] The intact preservation of DNA plays a significant role in digital data storage because it is closely related to data integrity. Figure 1 shows the schematic representation of the location of DNA preservation in the whole data storage process. Data in the range of kilobytes (KB) to megabytes (MB) have been stored in the DNA since 2012. Before the twenty-first century, less than tens of bytes of data that only contained texts have been attempted to be stored in DNA. Up to 659 KB of data containing texts, images, sounds, PDFs were successfully stored in DNA after the pioneering work of Church *et al.*[8] The limits of DNA digital information capacity can be attributed to two aspects. (1) Theoretically, if the binary codes of data are equally converted to four DNA bases, the probability is 0.25. The theoretical result is that one base corresponds to 2 bits. However, the probability distribution deviation, which is the difference between the frequency at which the base appears and the average frequency of 0.25, can reduce information capacity per base. For example, when the probability distribution deviation is 0.24, the corresponding information capacity can only reach ~1.14 bits per base.[9] (2) Mutual information that is the fidelity between writing and readout also determines the information capacity.

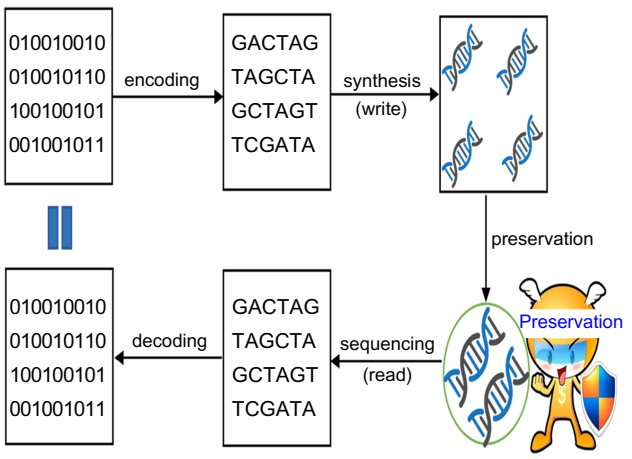


Figure 1. Schematic representation of the location of DNA preservation in the whole data storage process. Firstly, a computer algorithm maps strings of bits into DNA sequences (encoding). The DNA sequences are then machine synthesized (writing). After synthesis, the resulting DNA material can be stored by some preservation methods, such as being frozen in solution or encapsulated in a sealed and dried environment (preservation). Finally, automated sequencing instruments are used to generate a set of reads that correspond to the molecules they can detect (reading). DNA is decoded to recover the original information (decoding).

Generally, the transmission error rate (m_i), which means the probability that one base is incorrectly read out as one of the other three bases, is used to measure the fidelity between writing and readout. For example, when the m_i is 0.2, the corresponding information capacity can only reach ~0.07 bits per base. In summary, with the development of writing and reading technologies, the scale of stored data gradually increases.

The DNA used for information storage outperforms most traditional storage media, which is ascribable to its higher density, longer lifespan of hundreds of thousands of years, and lower energy costs.[10] Specifically, DNA storage is suitable for long-term backups of large amounts of data (cold data) due to the technical difficulty in random read access. That is to say, the data require no modification and have a few access opportunities. Until 2018, the largest data stored in DNA have exceeded 200 MB and were stored in the form of 13 million oligonucleotides.[11] One of the advantages of DNA data storage is an extremely high density (petabytes/gram). However, considering many practical factors, the theoretical density can hardly be reached. The reasons lie in the following three aspects. (1) DNA molecules should be preserved in specific conditions to slow degradation, such as dilute solution, silica. It implicates that the DNA loading efficiency (DNA weight/the total weight) cannot reach 100%, which causes the non-ideal storage capacity value. Therefore, how to improve the DNA loading efficiency in the preservation process is a focused research direction. (2) Due to the intrinsic limits of DNA synthesis technology, physical and logical redundancies inevitably reduce the actual density to a certain extent.[12] Also, the index used for addressing in the reading and sequencing process affects information density. It was calculated that the needed index ratio was 6.5% when using 200 base pair (bp) DNA molecules to store 1 Gigabyte (GB).[9] (3) The error in the process of synthesis and sequencing, the loss of DNA in the operation process, could reduce information storage density. Figure 2 shows the difference in the amounts of the stored data and the data storage

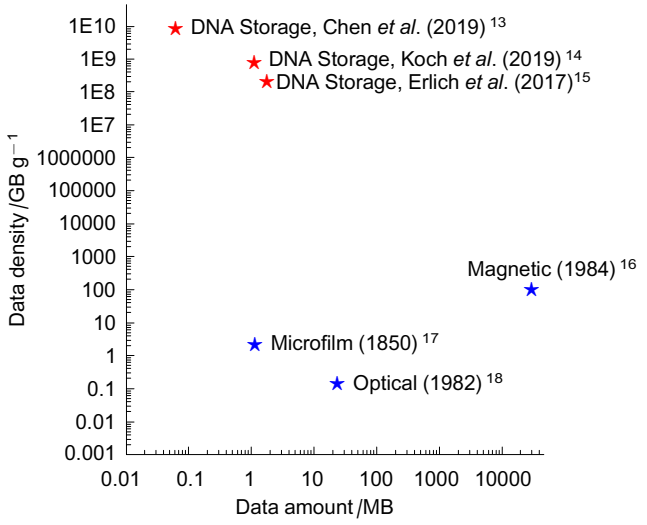


Figure 2. Amounts of data stored and storage densities achieved using DNA storage and the traditional storage approaches. The storage density is the total amount of information stored divided by the total weight of DNA used. The x-axis shows the total amount of data stored [13–18] (non-DNA-based data storage as control).

densities between some DNA data storage works and the traditional mediums. A long lifetime is another essential feature of DNA compared to other chemical stable encoded biopolymers. In contrast, DNA, if stored in an unprotected way, could be vulnerable to the depurination damage, hydrolytic cleavage, ultraviolet (UV) irradiation, oxidation. Consequently, how to protect and keep DNA safely for a long time to fulfill the requirements of DNA data storage appears significant.

In this review, we first analyze the influence of some factors, including intrinsic stability of DNA, environmental factors, and storage methods on the DNA long-term storage. Then, the benefits and disadvantages of diverse conservation approaches (*e.g.*, encapsulation-free, chemical encapsulation) are discussed. Among these methods, they are mainly classified into two kinds: the encapsulation-free method and chemical encapsulation. Finally, depending on oligo features in synthetic DNA databases, we provide advice for storing non-genetic information in DNA *in vitro*.

2. Influence factors of DNA degradation

In living organisms, the DNA structure is continuously changing due to cellular metabolites, UV radiation, oxidative damage, and exogenous DNA-damaging agents. However, the DNA damage response system that exists *in vivo* can recognize these damages, such as base damages, DNA backbone damages, and cross-links. Besides, DNA repair mechanisms[19] can ensure the integrity and contact information of DNA. On the contrary, DNA will degrade without these mechanisms due to intrinsic instability, environmental factors, and storage methods. Thus, there is a need to reveal the effect of these three main factors on the preservation of DNA. It is worth noting that the 'stability' in the text is a broad concept. The excellent stability of DNA in this review refers to the DNA with the normal secondary structure, intact base sequence information, and slow degradation rate.

2.1. Intrinsic stability of DNA

With the increasing development of DNA technologies, different forms of DNA have been discovered. Initially, the scientists found the double-stranded (ds) and the single-stranded (ss) structures, and then the triplet structure of DNA was found. For DNA used for data storage, it is usually a ss structure of 100 – 300 short nucleotides (nt).[20] The ss DNA structure is beneficial to be arranged geometrically, and later reassembled into the original data. The DNA with longer lengths can bring some issues, such as depurination, image drift, cost, and time consumption due to technological limitations of DNA synthesis. The more detailed DNA synthesis methods are described by Ceze *et al.*[7] This section will first focus on DNA duplex stability because it is the fundamental of DNA research and has been widely studied. It can be discussed in three aspects: (1) length and sequence; (2) base composition; (3) structure, furthermore, the stability of ss DNA can be discussed on the basis of ds DNA.

2.1.1. DNA duplex stability

Studies suggested that DNA duplex intrinsic stability depends on the length, base composition, sequence, and DNA structure. (1) Length and sequence. Generally, from the polymer view, DNA with a longer length has better stiffness when the length is beyond a certain threshold. (The certain threshold is defined as the persistence length, which quantifies the stiffness of long polymers.)[21] In most situations, the operation and preservation of DNA are performed in the solution. Therefore, it is more important to discuss the length dependence of duplex stability in the solution. In typical multivalent ion solutions, the helical structure of DNA can be stabilized by ions. In the solution of low ionic concentration, the neutralization becomes weak (the amount of bound ions is small) as the length of DNA helix increases, resulting in a decreased stability of DNA, which can be explained by the enhanced repulsive interactions between the charges (phosphate plus bound ion). On the contrary, the charge neutralization and ionic screening of the solution with high ionic concentration are much stronger, contributing to the relative decrease in the length-dependent effect.[22] For a given length, different DNA sequences of the same composition have different stability. Not only the hydrogen bonding between base pairs dictates DNA duplex stability, but the base stacking also plays a critical role in affecting overall duplex stability. The immediate neighbour of a base on a given strand will stack with that base and result in different stability. (2) Base composition. The G-C pairs (three pairs of hydrogen bonds) are stronger than the A-T pairs (two pairs of hydrogen bonds). Additionally, the DNA strands with more G-C pairs are more stable due to the more dominant stacking interaction of G-C.[23] (3) Structure. According to the characteristics of DNA molecules, the influence of DNA structure on stability can be summarized in three aspects: firstly, the alternating units of phosphoric acid and deoxyribose constitute the fundamental backbone of DNA, which improves the lateral stability; secondly, the internal stability provided by complementary base pairing. Thirdly, deoxynucleotide strands form a stable double helix structure, which significantly enhances the DNA stability.

2.1.2. Single-strand DNA stability

As mentioned above, the double helix structure plays a vital role in maintaining DNA stability. Therefore, the stability of the ss DNA without such a structure should be discussed when applied to data storage. Namely, a complementary base pair and a double helix structure do not exist in the ss DNA, and the single-stranded DNA has a coil conformation. Interestingly, the length-dependent effect of single-stranded DNA in the NaCl solution is slightly different. Due to the long-range nature of the repulsive Coulomb interactions, short DNA would become less stable with length at low NaCl concentrations. Such a destabilizing effect of double-stranded DNA is stronger than for single-stranded one.

2.2. Environmental factors

The previous studies have indicated that the storage environment of DNA has a significant impact on its stability. The environmental factors can be reduced to the following five aspects: temperature, water, UV irradiation, oxidation, and pH value due to the environment's complicated relationship.

2.2.1. Temperature

It is well known that the lower temperatures, the longer storage times. For example, the DNA of ancient permafrost samples can be preserved for $\sim$8000 years old.[24] As mentioned above, in the melting point (T_m) experiment, we can find that when the environmental temperature exceeds T_m,

the two strands of ds DNA dissociate into its constituent single strands. When it is lower than T_m, the local structure of DNA can be modulated.[25] Additionally, it is reported that DNA thermal stability is higher compared to other biopolymers in the absence of reactive oxygen species (ROS), ionizing radiation at room temperature.[26] However, when the temperature surpasses 140 °C, DNA integrity starts to be affected due to the increasing stacking free energy. Nowadays, there are various DNA storage methods, including cold storage at −4 °C, freezing DNA at −20 or −80 °C, cryopreservation in liquid nitrogen, and preservation at room temperature in solid state. Remarkably, the most common DNA storage approach for a long time is to freeze the DNA, including oligonucleotides and DNA biological samples, at ultralow temperatures (−20/−80/−192 °C). The lower temperature can inhibit DNA activities and slow down the chain segment motion. However, the cryopreservation of DNA could cause damage. In a related finding, it was reported that the DNA stored at either −20 or −80 °C in buffered solution degraded over time, whereas it remained stable when stored at 4 °C.[27] Some other studies discovered that the slower freezing of DNA was more harmful than the rapid freezing.[28, 29] We will discuss it in detail in the following (please refer to Section 3.1.3).

2.2.2. Water

Water can originate from both the storage environment and the DNA itself. However, amounts of previous studies have found that the dryness state of DNA and a lower degree of environmental humidity can facilitate the long-term preservation of DNA.[30, 31] It can be primarily attributed to hydrolytic damage, which will alter the structure of DNA. Specifically, hydrolytic cleavage mainly brings about single-strand breaks after depurination. Besides, it can cause base deamination that may result in the substitution of bases (*e.g.*, cytosine can be converted into uridine). Moreover, the relevant publication [32] has shown that when the activation energy is approximately the same, the equation for the decay kinetics of solid-state DNA is:

$$\frac{d_{c_{DNA}}}{d_t} = A \times e - \frac{E_A}{RT} c_{DNA} \quad (1)$$

where $d_{c_{DNA}}/d_t$ is the rate of change in DNA concentration over time (the decay rates of DNA), e is the base of the natural logarithm of the exponential function, E_A means Arrhenius-type activation energies, c_{DNA} represents the concentration of DNA. The A is an observed factor relevant to the preexponential Arrhenius factor and the effect of water. The equation also indicates that the lower temperature and dryness state are conducive to keep DNA safely for an extended period. Moreover, there are some slight differences in DNA stability between the gas and solution phases. The DNA in the gas phase is the DNA in the ultimate non-polar environment from which the intrinsic reactivity of DNA can be ascertained. Similarly, the DNA in the solution phase is the DNA mixed in some solutions such as a NaCl solution or phosphate-buffered solution in which some ions can stabilize DNA molecules. In solution, the hydration effects and the interactions with ions can cause a slight difference in DNA stability from the gas phase. The melting point can be used to characterize the stability of a DNA duplex in solution, which is mainly subjected to base pairing and base stacking. Among them, the base stacking interaction is dominant. However, the stability in the gas phase has a weak correlation with one in solution when DNA has a different GC content. This is due to the occurrence of a relatively stable mode, in which the water molecule is coplanar with G-C, and the difference in the relative stacking of DNA between non-polar and polar media.[33]

2.2.3. UV irradiation

When UV light reaches DNA, the generated excited electrons with energies below the ionizing energies can induce DNA strand breaks, cross-linking, dimer formation and nucleobases modification. These low-energy electrons cause DNA structure changes, removal of molecular groups (*e.g.*, phosphate groups), and reduced conductivity. Generally, the UV irradiation has both direct and indirect mechanisms depending on the UV wavelength. Directly attacking DNA bases leads to the most severe damage due to the absorption of short-wavelength UV photons by bases. DNA can also be indirectly damaged when exposed to long-wavelength UV light through the production of ROS.[34] The most simple and efficient method for preventing DNA from UV light *in vitro* is storing DNA in the shade or adding UV absorbers to improve DNA photoresistance. Recently, it was reported that DNAzymes can be used as DNA protectors. Barlev and Sen [35] demonstrated that guanine and its oxidation product, 8-oxoguanine, can repair a photoactivated cyclobutene thymine dimer. The cyclobutene thymine dimer is the most common chemical DNA damage upon exposure to UV light.[35] The author designed a DNAzyme (UV1C) with its guanine quadruplex catalytic core to implement a self-preservation mechanism when DNA was exposed to UV light. In addition, the previous studies also confirmed that G-quadruplex DNA is an intrinsically stable secondary structure, which is connected by G-G base pairs.[36] These discoveries can prove that DNA may be specially designed to protect itself when subjected to UV light in the future.

2.2.4. Oxidation

Outside the organism, attacks on deoxyribose and bases induced by oxidation can contribute to chain breaks, formation of abasic, and base modification,[37] respectively. Reactive oxygen species, which were the main factor of DNA degradation during storage, was also observed in the solution containing metal ions or lipids due to triplet oxygen and water production. Hydroxyl radicals are one of the crucial reactive oxygen species. As is known, DNA is likely to be degraded by hydroxyl radicals, the production of which is often catalyzed by transition metal ions. Evans *et al.*[38] have indicated that the stability of DNA in either of the demetalated buffers was significantly higher than the DNA in the nondemetalated buffers. Also, hydroxyl radicals are discovered to abstract hydrogen atoms from several sites in the deoxyribose sugars, causing conformational changes of DNA. Some formulations, such as diethylenetriaminepentaacetic acid and ethylenediaminetetraacetic acid, are known to strongly inhibit hydroxyl radical production mediated by bound metal ions, thereby improving the stability of DNA in solution. If biological DNA samples cultured in the medium or nonbiological information encoded in DNA are preserved *in vitro*, artificial protection measures are required. Gudkov *et al.*[39] reported that unmodified hydrated C-60 fullerene molecules can inhibit the formation of ROS in water and 8-oxoguanine in DNA, Grass and co-

workers[26] suggested that leveraging amorphous silica as a barrier can protect DNA from ROS and heat.[26]

2.2.5. pH Values

Yakovchuk *et al.*[23] showed that the stacking free energy decreased with increasing salt concentration in solution. That is likely because more efficient screening of phosphate charges reduced electrostatic repulsion between DNA molecules. Besides, it is a matter of fact that a neutral or slightly alkaline pH is suitable to preserve DNA[5] because the alkaline conditions can the degradation pathway *via* the acid-catalyzed, water-independent N-glycosidic bond cleavage during long storage time. It is well established that the structure of DNA is 'undeformed' at pH ~5–9 because purine and pyrimidine bases, the phosphoryl chain, and the sugar ring keep intact and well-linked. However, the electrostatic interaction between the protonated amino groups in the imperfectly bonded area and solvent-mediated swelling could contribute to the H-bond breakage and continuous proton uptake at low pH.[40] Thus, the final structure of DNA could be an internal solvated expanded helix that is likely stable in solution at high ionic concentration due to the screening of the oppositely charged electrolyte ions. The internal solvation and expansion, as described for the acid-deformed DNA, could appear at high pH (pH > 12), which can be attributable to the formation of corresponding guanyl and thymyl anions caused by proton dissociation of their enol groups.[41]

2.3. Storage methods

DNA degradation did occur for any sample regardless of storage methods. With the advanced optimization of preservation approaches, the rate of degradation can be gradually minimized. The previously developed DNA protection schemes included solution storage, dry storage, freeze-drying storage with stabilizers, and DNA encapsulations. These storage methods can provide different protective effects for DNA, such as storage time, DNA integrity. They are evaluated in another Section below.

3. Evaluation of different preservation methods

A variety of methods have been proposed to store DNA for addressing the issue of DNA degradation. They can mainly be classified into two categories: the encapsulation-free methods and the chemical encapsulation approach. Table 1 presents a comparison of half-lives of DNA stored by each method. Table 2 summarizes the relative advantages and disadvantages of different methods. Additionally, some containers for DNA preservation are mentioned in this review. Finally, the research world of DNA data storage needs a complete automation device,[42] including encoding, writing, synthesizing, storage, reading, and decoding procedures.

Table 1. Quantitative comparison of storage methods.

Storage method	k_0 /s^{-1} nt^{-1} [a, b]	$t_{1/2}^{158nt}$/year [c, d]	Ref.
Solution preservation	1.74E-10	0.8	43
Dehydration	3.3E-12	42	38
Freeze-drying (−5 °C)	4.7E-15	~30 000	44
Only additives	1.1E-11	12	37
Chemical encapsulation	1.4E-12	100	32

[a] All degradation rates correspond to the temperature of 20 °C except the freeze-drying; [b] k_0 represents the degradation rate per second per nucleotide; [c] $t_{1/2}^{158nt}$ represents the half-lives of DNA of 158 nucleotides; [d] the half time per nucleotide is $t_{1/2}^{nt}$ (20 °C) = ln(2)/k_0. For comparison, the half-lives of DNA of 158 nucleotides is $t_{1/2}^{158}$ (20 °C) = $t_{1/2}^{nt}$ (20 °C)/158.

3.1. Encapsulation-free methods

Encapsulation-free methods primarily consist of solution preservation, dehydration, lyophilization, and addition of additives.

3.1.1. Solution preservation

The most common approach for biological sample preservation (*e.g.*, DNA, RNA, proteins) in the laboratory is solution storage,[45, 46] which can prevent chemical and enzymatic degradation. Initially, DNA was stored as a precipitate in ethanol. Subsequently, an ammonium-based ionic liquid can offer improved concentrations and preservation stability for DNA due to the hydrogen bonding between the ionic liquid and DNA.[47] In the presence of chemical analogs of microbial auto-regulators, related studies on preventing DNA degradation in aqueous solutions have proved the mechanism by which alkylhydroxybenzene protects DNA.[48] Preparation is faster for the solution, whereas DNA is vulnerable to depurination, depyrimidination, deamination, and hydrolysis due to the presence of water. Also, DNA should be isolated from ethanol prior to use (*e.g.*, isolation, transferation), which is detrimental to frequent reading and sequencing process in the application of DNA data storage. The storage stability in solution at most lasts for ~1 year, which is not enough for fulfilling the required lifespan of the DNA data preservation (>1000 years). As mentioned above, if one needs to store DNA using solution preserva-

Table 2. Relative advantages and disadvantages of different methods.

Storage method	Solution	Dehydration	Additives	Freeze-drying method	Emulsion encapsulation	Nanoparticle-based
Advantages	Fast and easy preparation			Better integrity	Better protection and data management	
Disadvantages	Hydrolysis damage	Denaturation damage	Short shelf-life	Freeze-thaw damage, high cost	Low loading density	Template dissolution influence
Impact on data storage	Rapid loss of stored information			Frequent sequence, decoding errors	Low-density data storage	Disruption of stored data
Applicability for DNA [a]	Plasmids	Oligonucleotides	Plasmids	Plasmids	ds DNA	Artificial DNA

[a] These DNA preservation methods can be applied for any form of DNA, but the table states the main application.

tion, it is better to preserve DNA in alkaline environments to inhibit the primary degradation pathway *via* the acid-catalyzed, water-independent cleavage of the N-glycosidic bond. Moreover, the ionic strength is a vital factor for the stability of DNA due to the effects of depurination and base-catalyzed β-elimination. Simultaneously, the enhanced oxidation, which is the primary threat of DNA degradation in solution, can lead to base oxidation and strand breaks. In some rare cases, DNA—DNA cross-linkages induced by oxidation have been reported.

3.1.2. Dehydration

It is a matter of fact that solid-state DNA is more stable than DNA in solution due to the reduced molecular mobility and removed residual water that can accelerate degradation reactions.[49] Several methods of removing water from liquid preparations can produce dehydrated DNA formulations (*e.g.*, spray drying, air drying). However, the desiccation processes can lead to the loss of DNA structural stability. One of the potential impact factors is the shear stress in spray drying procedures, which induces structural damage. The other factor is that dehydration can lead to changes in the secondary structure of DNA. Some studies indicated that only very long DNA strands are susceptible to shear stress when mentioning shear-induced damage. Thus, the oligonucleotides in data storage are not vulnerable to damage.[50, 51] As mentioned previously, the dehydration likely contributes to conformational changes (between 92% and 70% relative humidity).[52] The denaturation of DNA was further discovered when relative humidity was below 70%–50%. Spectroscopic methods have observed the destacking of the bases and fragmentation of hydrogen bonds. However, more studies have reported that the denaturation induced by dehydration can be recovered upon rehydration except for the heated dry-state DNA.[53] Notably, there are many spectacular examples of successful amplification and sequencings from ancient specimens. For instance, the mitochondrial genome sequence of a middle Pleistocene cave bear can be reconstructed by utilizing an improved silica-based extraction protocol in combination with ssDNA library preparation techniques.[54] The ultra-short DNA fragments, oxidized and hydrolyzed bases in ancient remains have been discovered because the ancient DNA would suffer from microbial infestation and post-mortal organic substances compared to the dehydrated DNA in modern preservation technology. Although pronounced molecular weight changes of dried, naked DNA stored at 0–4 °C for 6 months were confirmed,[55] adding additives [*e.g.*, trehalose, polyvinyl alcohol (PVA), biomatrica] can stabilize dry-state DNA to provide extra protection.[56] Furthermore, the synthetic oligonucleotide of DNA data storage can tolerate a certain degree of errors or missing data during storage time due to error correction codes.[57]

3.1.3. Freeze–drying

Freeze-drying, also called lyophilization, has long been used for keeping the long-term stability of biological samples.[58, 59] The DNA samples can be well stored to avoid many environmental factors like high temperature and oxidation, which remarkably damage their structural integrity under the joint protection of drying and freeze. However, the effects of freeze-drying on synthetic DNA integrity have yet to be explained clearly. The first study of successful DNA conservation is that calf thymus DNA can maintain its integrity at −192 °C.[40] Subsequently, gel electrophoresis and AFM showed that the DNA nanostructure carrier could maintain structural integrity after the freeze-thaw process.[60] Besides, freeze-dried mammalian cells also can maintain their DNA integrity,[31] Previous studies have shown that frozen DNA solutions in the buffer are less stable than those stored at 4 °C,[27] and that DNA formulations are degraded after single or multiple freezing cycles.[61] A rational explanation for the abnormal result during the freeze-thaw process may be that it is a consequence of mechanical shearing damage to DNA by ice crystals during freezing. The longer the intermediate state between the ice crystal state and the aqueous solution lasts, the greater the ice crystal damage to DNA integrity. Despite the fact that lyophilization can provide sufficient protection, the high salt concentration could promote mismatches between bases and nonspecific adsorption[62] during the lyophilization procedures. Besides, the expensive cost of maintaining the frozen state, which is estimated to surpass 54 million in 2007 (Ref. 40) in the United States, should be considered.

3.1.4. Addition of additives

Additives, also called stabilizers, have the ability to promote glass formation, which can provide additional protective effects on DNA stability.[63, 64] The most common stabilizer is trehalose. It is derived from anhydrobiosis of some small animals, such as brine shrimp and tardigrades. These invertebrates can survive long in a suspended animation state after dehydration due to the sugar trehalose production. Trehalose, a non-reducing disaccharide, can form tight hydrogen bonds with phosphate groups and interact with some polar groups upon dehydration to achieve the protected effect of DNA molecules. Precisely, trehalose can screen strong phosphate–phosphate negative charge repulsion between DNA molecules and stabilize the base stacking of DNA during and after dehydration.[65, 66] Zhu *et al.*[67] have demonstrated the stabilization effect of trehalose on the three-dimensional structure of calf thymus NaDNA in the dry state. Studies showed that the fragmentation of longer DNA regions into shorter ones is the main reason for most physical property changes during DNA degradation and can be monitored by polymerase chain amplification (PCR)-based methods. Trehalose can only be sufficient for short-term storage, according to the work by Ivanova and Kuzmina.[56] The diluted DNA was preserved with trehalose at room temperature for a month and only reached 46% PCR success. In order to validate PCR success, samples were sequenced, and the results are consistent with PCR success. The non-toxic PVA is another ideal candidate for DNA preservation. It forms strong hydrogen bonding between DNA and PVA and keeps some amounts of water molecules tightly attached even in dehydrated solvents. Ivanova and Kuzmina.[56] found that after 2 years of storage, Tris-buffered PVA provided adequate protection (50% PCR success for diluted DNA) at room temperature but did not offer sufficient protection at 56 °C (complete degradation for diluted DNA). On the contrary, trehalose was more efficient at 56 °C (17% PCR success for diluted DNA). Therefore, compared to trehalose, PVA is more appropriate for long-term storage at room temperature. Recently, some synthetic preservation matrices have been developed to offer better protection for biological samples. DNAstable™ is one of the commercially available synthetic polymers that mimic the natural molecular principles of anhydrobiosis by forming a thermo-stable barrier around

the biological sample to protect it from degradation. In short-term and long-term storage experiments, DNAstable™ can provide better DNA protection at 56 °C (33% PCR success for diluted DNA) and room temperature (52% PCR success for diluted DNA) after 2 years of storage.[56] Overall, trehalose might be sufficient for short-term storage, but PVA and DNAstable™ can be recommended for long-term storage. We do not recommend PVA for long-term storage at high temperatures or in some tropical countries. While DNAstable™ offers the best protection, both trehalose and PVA can be utilized as cost-efficient alternatives. Table 3 summarizes the comparative analysis of these three additives. Besides, one study has confirmed that adding carrier nucleic acid to the DNA standard solution can improve the stability of the plasmid DNA in solution stored frozen based on the 'self-protection' of concentrated DNA solution during freeze-thaw. However, we should also notice that the problem in DNA purification resulted from stabilizing additives. There are two primary forms of adding additives. One is to mix the DNA with the additive solution, and another is to precoat each of the 96 wells with the

Table 3. Comparison of three commonly used stabilizers.

Stabilizer	Advantages	Disadvantages	Ref.
Trehalose	Cheap, less hygroscopicity	Short-term storage	67
PVA	Nontoxicity, long-term storage	Thermolability	57
DNAstable ™	Best protection	Relatively expensive	30

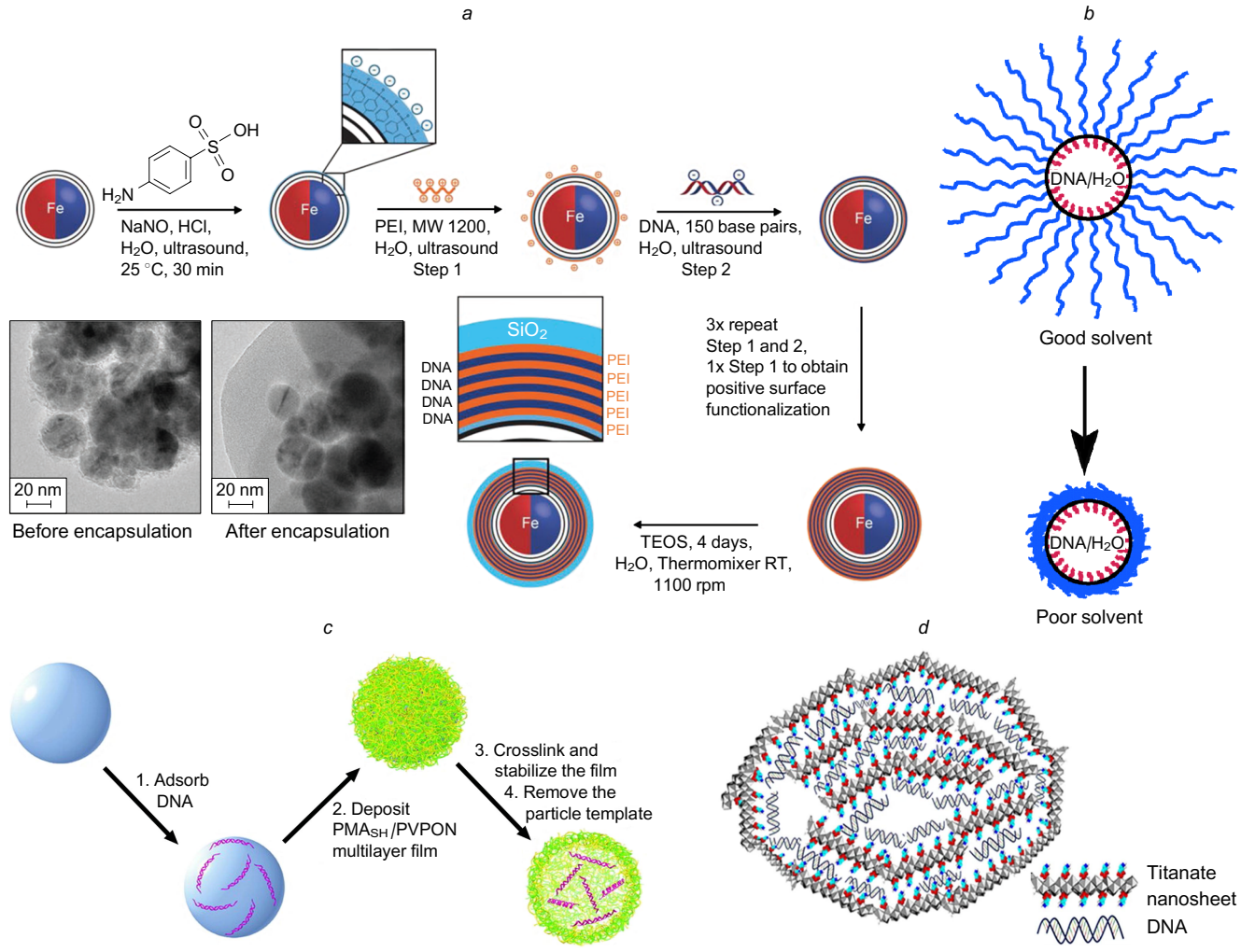


Figure 3. Schematic representation of some chemical encapsulation methods: (*a*) schematic of layer-by-layer DNA loading on Fe/C nanoparticles. In the first step, the Fe/C nanoparticles are functionalized with sulfanilic acid. Particles are then immersed in a solution of polyethyleneimine (PEI) to introduce a positive surface charge. After a washing step, DNA can be immobilized onto the surface. This procedure can be repeated multiple times before the particles are immersed in a solution with tetraethyl orthosilicate (TEOS). TEM images of nanoparticles before and after encapsulation were taken to confirm silica growth. Reprinted from Ref. 13. Copyright 2019 John Wiley and Sons; (*b*) schematic representation of an emulsion droplet in a good (top) and poor (bottom) solvent for the hydrophobic chain part. The change of the solvent quality results in a collapse of the interfacial polymer layer. Reprinted from Ref. 71. Copyright 2005 American Chemical Society; (*c*) Encapsulation of DNA into degradable polymer capsules. Adsorption of DNA onto amine-functionalized silica particles: (*1*) is followed by the assembly of a thin polymer film prepared *via* the alternating deposition of thiol-modified poly(methacrylic acid) (PMA_{SH}) and poly(vinylpyrrolidone) (PVPON); (*2*) oxidation of the PMA_{SH} thiol groups into bridging disulfide linkages; (*3*) removal of the core particles; (4) result in stable polymer capsules. DNA chains are confined within the capsule interior and are released in a reducing environment. Reprinted from Ref. 76. Copyright 2007 American Chemical Society; (*d*) hybridization of a cationic (3-aminopropyl)triethoxysilane (APTES)-anchored titanate nanosheet with DNA. Reprinted from Ref. 82. Copyright 2016 Scientific Reports.

additive and then add the dry-state DNA. Thus, one can resuspend DNA samples in water to purify DNA from the first method. Similarly, the second DNA sample can be rehydrated with nuclease-free water to purify. Because the effects of trehalose on polymerase processivity have not been verified, each PCR reaction in literature would contain a certain volume of trehalose to offset trehalose effects.

3.2. Chemical encapsulation methods

Apart from the chemical solution methods convenient for the conservation of DNA, the chemical encapsulation methods that can provide longer shelf-life and better protection have attracted a great deal of research interest. In the beginning, the encapsulation of DNA was widely applied in gene therapy and DNA delivery,[68,69] which transported the drug to active sites through some hostile medium on the way to the cell nucleus. The polymer microcapsules can be prepared using the emulsion method [70,71] and nanoparticle-based encapsulations.[72–75] Concerning the emulsion method, the encapsulation of DNA in calcium alginate nanobeads using the phase inversion temperature emulsification method can provide a good environment for the storage of DNA (Fig. 3 *b*).[71] The nanoparticles-based encapsulations included the absorption of DNA onto the surface of particles. This is followed by adding a protective layer on the top of nanoparticles or utilizing the encapsulated materials to prevent DNA from external damage. Zelikin *et al.*[76] encapsulated DNA with varied size and conformation (*e.g.*, ss, linear ds, and plasmid DNA) within the interior of polyelectrolyte capsules. These microcapsules can be stored at 4 °C for several weeks without signs of capsule degradation (Fig. 3 *c*). The ancient biological fossils were the well-known examples to encapsulate DNA by natural biological molecules (*e.g.*, apatite, collagen structures [77] and crystal aggregates [78]), which protect DNA from external environmental damage. Intrigued by these encapsulation methods and natural fossils, Grass and co-workers [79] proposed a synthetic DNA silica fossilization technology that mimics the hermetic diffusion barrier of fossils. Accelerated aging tests compared the DNA stability of this silica encapsulation method with the other three dry storage methods.[80] The DNA encapsulated in silica showed the best DNA preservation as well as ROS-resistance and heat-resistance. Other encapsulation applications, for instance, DNA nanostructure with graphene encapsulation, can promote the thermal stability of DNA. Figure 3 demonstrates some chemical encapsulation methods.[81]

As mentioned above, because nanoparticle-based encapsulation encompassed loading DNA onto the surface of these particles, we will discuss some detailed DNA loading methods. Several loading methods have been developed depending on the surface chemistry, including covalent binding and non-covalent attachment (Fig. 4). The latter mainly involves electrostatic adsorption and other forms of adsorption.[83]

3.2.1. Covalent attachment

DNA covalent attachment, which can provide a strong linkage between DNA and particles, involves dative bonds and covalent bonds. The dative bonds can directly conjugate DNA to the surface of nanoparticles, whereas the covalent bonds link DNA *via* the interaction between surface ligands and DNA. The chelation of metal ions and Au–thiol linkage are most frequently used in dative bonds. The metal ions, especially polyvalent metal ions, can promote DNA adsorption on nanoparticles. For example, divalent Ca^{2+} was found to bridge DNA and polydopamine (PDA).[84] Using some transition metals can further improve binding efficiency between DNA and PDA due to the rich metal-chelating groups on PDA.[85] There are also some other metal-mediated DNA adsorptions,[86] such as Mg^{2+}. Other than the metal ions coordination, the interaction of thiol groups with the surface of AuNPs can be the predominant basis of AuNP bioconjugation with DNA. Notably, the attachment strength can be tuned *via* multidentate thiols, such as promoting the linkage strength by using bidentate thiols [87] and giving colloidal stability to quantum dots *via* tetradentate thiol ligands.[88] However, thiol chemistry has the limitation of an additional thiolating step of DNA. This procedure is complicated and gives extra demand for raw DNA materials. Meanwhile, it would produce toxicity due to low-compatibility of the thiol groups in many bio-systems. Besides, one can utilize classical bioconjugation chemistry for covalent chemical attachments such as click chemistry, carbodiimide chemistry, and other chemistry couplings. Wang *et al.*[89] demonstrated that

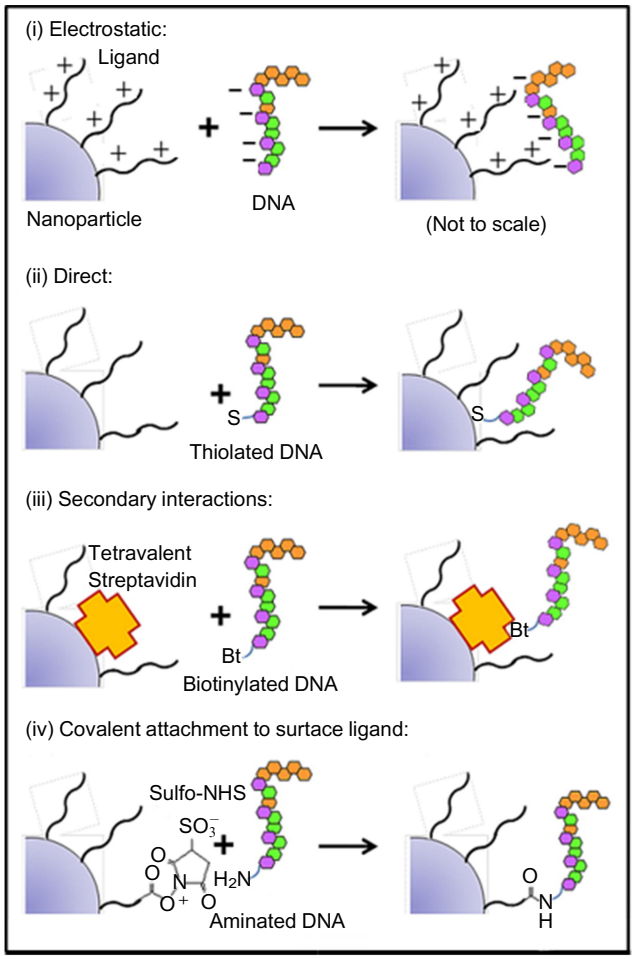


Figure 4. The covalent and non-covalent attachment used for bioconjugation to NPs as a highlight by the use of DNA: (i) electrostatic interaction, mediated charge-by-charge-based assembly; (ii) dative bonds, the interaction of free thiols with the surface of AuNPs; (iii) secondary interactions, biotin–streptavidin binding; (iv) covalent chemical attachment, for example, N-hydroxysulfosuccinimide (NHS-) and maleimide-mediated conjugation to amines and thiols. Reprinted from Ref. 83. Copyright 2013 American Chemical Society.

metal-organic framework nanoparticles (MOF NPs) functionalized with a layer of azide-modified polyethylene glycol (PEG) polymer can bind to a diarylcyclooctyne-modified DNA through cooper-free click chemistry. Li *et al.*[90] illustrated that DNA can be loaded on plasma-treated multilayer carbon shell-encapsulated gold nanoparticles *via* carbodiimide chemistry. Compared to thiol chemistry, these DNA immobilization methods can provide stronger binding and better biocompatibility. It is worth noting that the covalent attachment of DNA to functionalized nanoparticles or other carriers requires some modifications. Although oligonucleotides with terminal amino, thiol groups, and terminal alkynes can be obtained *via* post-synthetic modification, the modifications are introduced during automated oligonucleotide synthesis in most cases. Thus, covalent attachment to the solid support is rarely an option for wild-type DNA.

3.2.2. Non-covalent attachment

Non-covalent attachments, which appear to be a comparatively weak binding compared to covalent binding, popularize them for many preparations and applications due to their simplicity, cost-effectiveness, and reversible binding. They are also termed as self-assembly because the preparation procedures only need stoichiometric mixing of the two chemical components in most cases. A well-known example of non-covalent attachment is electrostatic interaction, which means that charged NPs electrostatically attract oppositely charged DNA. It is well-established that DNA has a strong negative charge due to the phosphate backbone when the solution pH is above 2. Therefore, DNA can be loaded onto positively charged NPs *via* electrostatic attachment. B.Liu and J.Liu[91] showed that indium tin oxide (ITO) nanoparticles possess high DNA adsorption capacity at low pH because of the positively charged surface of ITO. Such interactions are quite sensitive to the pH value, ionic strength, and concentration of components. Zhang *et al.*[92] demonstrated that the electrostatic interaction between DNA and citrate-capped gold nanoparticles can be tuned by salt concentration and pH to increase DNA adsorption capacity. Recently, layer-by-layer (LBL) electrostatic complexation has become of paramount importance among the electrostatic interaction of non-covalent attachment. We will discuss it in detail (refer to the next Section). Other non-covalent chemistries, including hydrophobic–hydrophilic and host–guest interactions, can be applied in the DNA loading.[93] Secondary interactions like biotin–streptavidin interaction can be utilized to bind DNA and NPs since modified DNA with biotin groups can couple to avidin-labeled NPs.

3.2.3. Layer-by-layer self-assembly

These DNA storage and loading methods mentioned above can provide efficient protection of DNA, but the low DNA loading remains a problem that deserves serious consideration. Although Barrett and Edgar[94] used diamond chips to preserve high-density DNA, they can only achieve short-term storage. Another example is the physical storage of a high-density DNA data storage library by storing dehydrated DNA spots on glass.[95] Additionally, the protection of DNA by highly stable layered metal oxide was developed by Kim *et al.*[82] In their study, the cationic APTES-anchored titanate nanosheet served as a reliable long-term nanocontainer of DNA against acidic corrosion and UV-Vis irradiation, and it was ascribable to the titanate nanosheets of high chemical and thermal stability (Fig. 3 *d*). Importantly, the present DNA-layered titanate nanohybrid can store 9.3 wt.% of DNA. Instead, the SiO_2 nanoparticle-based hybrid system was reported to only show the DNA storage capability of 2.5 wt.%. However, these methods are complicated processes, and metal ions may bring extra damage, such as enhanced oxidation. The LBL self-assembly promises to be further developed with the advantages of the high efficiency of encapsulation, the feasible design of the structure as well as the properties of a protective shell. The LBL self-assembly technique is initially applied to alternatingly deposit the polyelectrolytes with opposite charge onto a flat charged substrate by repeated dip-rinsing cycles through electrostatic interactions.[96] Beyond the use of a flat substrate and common polyelectrolytes, NPs and biomolecules (such as DNA) can be used as the LBL self-assembled components. Thus, it is convenient to achieve higher DNA loading levels through LBL self-assembly due to the formation of multilayer structures. Taken together, to implement high DNA loading with long-term protection, we can utilize the LBL self-assembly incorporated DNA preservation methods. Grass and co-workers[13] proposed to use alternating adsorption of DNA and polycationic molecules on magnetic nanoparticles with a protective silica layer on the top of the multilayered nanoparticles to screen the DNA from external environmental damage[13] (Fig. 3 *a*). Simultaneously, DNA weight loading can achieve 7.8 wt.% compared to 2.5 wt.% for the SiO_2 nanoparticle-based hybrid system. Despite the combination of several preparation technologies that can achieve better preservation and high-density compaction, the core dissolution may damage the nucleic acid chains, leading to incomplete functionality. If the reaction conditions are not mild, DNA chains are prone to be fragmented under shear. Furthermore, the treatment with some chemical reagent (*e.g.*, hydrofluoric acid (HF), HCl, and chloramine T) may affect the DNA intact function. Although some research results showed that DNA can retain enzymatic functionality after the encapsulation process and suggested that the encapsulated DNA can exhibit the ability to preserve $\sim$90% of the helicity, the investigation into the sequential integrity or activity of released DNA should go deep. Finally, it is worth noting that DNA is released from the carriers after storage. The most common practice of DNA disassembly after the storage is incubation with standard elution buffers and salt solutions (*e.g.*, phosphate solution and NaCl solution). The experimental results revealed that standard elution buffers and salt solutions did not successfully release a major portion of the surface DNA due to the strong binding of the polycation to the DNA backbone. Thus, a more efficient method is to add a polyanionic molecule. The idea is to introduce a stronger ionic interaction between the polycation and the polyanionic molecule, which releases the deposited DNA. Adding an excess of the polyanionic molecule should not interfere with the DNA since the excess negative charge would only lead to repulsion. The amount of DNA released using a polyanionic molecule was four times higher compared to applying the individual salt solution.

3.2.4. Other aspects of preservation

Except for the mentioned-above DNA preservation methods, determining the appropriate DNA storage containers is also crucial. Currently, most containers, like filter paper for preservation,[97] airtight stainless-steel mini capsules, 96-well

plates [98] are applicable for biological samples. Hence, density and cost are compromised that are unfavourable for data storage. Additionally, DNA-based data storage within a cell, also called *in vivo* storage,[99] was used as a DNA carrier system due to its high fidelity during cellular replication and well-preservation after cell death. The *in vivo* storage is suitable for collecting new data as biological recording devices compared to *in vitro* storage that is more practical because of its cost, scalability, and stability. The *in vitro* storage promises to be a priority research area in terms of the synthetic DNA used in data storage. However, in the future, we need an automated DNA data storage device, including the whole process of encoding, synthesizing, storage and read/decoding with minimal human labour and interference.

4. Conclusion and outlook

Data storage is a promising alternative to replace traditional mediums, which are limited by their storage density. Simultaneously, the gradual loss of stored information would occur if DNA molecules were degraded naturally without any protection measures. Most of the early DNA preservation technologies are focused on biological DNA samples or forensic samples. There is not yet an optimal method for DNA data storage. This review is mainly based on the factors affecting DNA degradation and describes the advantages and disadvantages of various protection methods. Solution preservation is detrimental to synthetic oligonucleotides due to the damage of hydrolytic cleavage, depurination, depyrimidination, and deamination. Techniques such as dehydration and freezing can mitigate water influence on degradation and reduce the molecular mobility of DNA to slow down degradation. Adding additives can bring an extra protective layer. However, it is crucial to provide a long-term stable environment for synthetic DNA-encoded nonbiological information. Recent implementations related to enacapsulation of DNA by polymers, inorganic substances, or metal oxide materials provided a protective barrier against chemical, UV light, and oxidative attack. However, most encapsulation methods are applied in gene therapy or drug delivery that cannot achieve high-density loading. Synthetic DNA used in data storage should combine with the layer-by-layer assembly to facilitate high-density loading and efficient long-term protection of DNA. For information stored in DNA indefinitely (such as archives in libraries), the combination of freeze-drying and adding additives can maintain the long-term stability of the encapsulated DNA under existing conditions. The following problem is how to reduce the cost of maintaining a frozen state and overcome the challenges to scale. Based on the features of oligo in synthetic DNA databases, such as (1) synthesis of several 100 ~ 300 nt short DNA fragments encoding digital information; (2) unordered in large pools; (3) the majority need of long-term storage (> 1000 years), we must evaluate a suitable preservation method to satisfy the requirements of safekeeping. Hence, we conclude some preservation advice for synthetic oligos: (1) DNA stored in the solid-state is more stable compared to liquid storage; (2) dehydration and encapsulation are more practical for inexpensive long-term DNA preservation on large scale; (3) the use of additives can provide additional protection; (4) frozen samples bring expensive cost, but better protective efficacy; (5) incorporating loading technologies (*e.g.*, LBL assembly) enable DNA safekeeping with high-density loading. We expect that these preservation suggestions can promote further research that may extend the lifetime of DNA storage. Advances in DNA storage technology allow long-term protection of data stored in DNA at room temperature, making it suitable for long-term backup of large amounts of data.

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The following designations and abbreviations are used in the review:

APTES — (3-aminopropyl) triethoxysilane,
bp — base pair,
ds — double-stranded,
GB — gigabyte,
HF — hydrofluoric acid,
ITO — indium tin oxide,
KB — kilobyte,
LBL — layer-by-layer,
MB — megabyte,
MOF — metal-organic framework,
NHS — *N*-hydroxysulfosuccinimide,
NPs — nanoparticles,
nt — nucleotides,
PCR — polymerase chain amplification,
PEG — polyethylene glycol,
PEI — polyethyleneimine,
PMA_{SH} — thiol-modified poly(methacrylic acid),
PVA — polyvinyl alcohol,
PVPON — poly(vinylpyrrolidone),
ROS — reactive oxygen species,
ss — single-stranded,
TEOS — tetraethyl orthosilicate,
UV — ultraviolet.

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