Nanopore-Based DNA Hard Drives for Rewritable and Secure Data Storage

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ABSTRACT

Nanopores are powerful single-molecule tools for label-free sensing of nanoscale molecules including DNA, which can be used for building designed nanostructures and performing computations. Here, DNA hard drives (DNA-HDs) are introduced based on DNA nanotechnology and nanopore sensing as a rewritable molecular memory system, allowing for storing, operating and reading data in the changeable three-dimensional

structure of DNA. Writing and erasing data are significantly improved compared to previous molecular storage systems by employing controllable attachment and removal of molecules on a long double-stranded DNA. Data reading is achieved by detecting the single molecules at the microsecond timescale using nanopores. The DNA-HD also ensures secure data storage where the data can only be read after providing the correct physical molecular keys. Our approach allows for easy-writing and easy-reading, rewritable and secure data storage toward a promising miniature scale integration for molecular data storage and computation.

KEYWORDS: Single-molecule, Nanopore sensing, DNA nanotechnology, DNA nanostructures, Molecular data storage

DNA is a natural material for storing genetic information, but it has also been repurposed as building blocks for creating designed nanostructures with bespoke functionality. Nanoscale structures and patterns can be made by using the self-assembly

of single-stranded DNA tiles or by stapling a scaffold DNA strand into a designed structure using complementary short single-stranded oligos.[1-3] Traditionally, DNA constructs and their shape have been characterized using Atomic force microscopes (AFMs), transmission electron microscopes (TEMs) and optical methods.[4] For scalable label-free characterizing a large number of single molecules made from DNA, nanopore sensing is an ideal method.[5] Crucially, the ionic current signal of a molecule passing through the nanopore reveals its 3D shape and thus translates DNA structures into electrical signals.[6-9] The combination of tailor-made DNA nanostructures with the ease and rapidity of nanopore analysis of these structures shows great potential for applications in biosensing, molecular machines and molecular data storage.

For molecular data storage, the most common approach is to store data in the DNA sequence by synthesizing DNA strands with designed sequences,[10] with obvious advantages like high density, high durability and low maintenance costs.[11-15] However, synthesis-based DNA data storage relies on creating new DNA strands with unique sequences for writing more data, causing intrinsic disadvantage in the ease and cost of

data writing. Besides, DNA with a fixed sequence is hard to modify, creating a serious challenge for data operations and computation akin to classical computer memory. Data security and privacy are also major concerns of important archival storage. Encryption in the physical keys using the unique characteristics of DNA molecules will allow for secure storage as the information is only retrievable with the correct key.[16,17] The flexibility of DNA as a functional material based on Watson-Crick base pairing opens pathways to address the above questions. Strategies[18,19] based on DNA nanotechnology were put forward to make full use of the advantage of the material based on base pairing and toehold mediated strand displacement.[20-22]. For instance, employing existing native DNA strands as a carrier for information provides alternative routes to data storage in direct DNA sequence, including the DNA punch card method[23] or the catalog DNA method[24]. Similarly, data can also be stored in the 3D structures of designed DNA objects,[9,25,26] opening a new route with the potential of convenient writing and reading as an alternative to the data storage in the DNA sequence.

Here, inspired by the principle of hard disk drive where data is stored and changed by operating on the sites on the disk, we dramatically improve the utility of DNA nanostructures to achieve rewritable and secure data storage by adding and removing molecules in domains along a DNA strand. We name our system DNA hard drive (DNA-HD), which is easily operated requiring only the mixing of premade molecules. Using our DNA-HDs, we can write data by attaching molecules on dsDNA and erase data by their targeted removal. We will demonstrate the full capabilities by a complete writing-erasing-rewriting cycle in this paper. Also, the data can be easily read with nanopores by passing the molecules through the nanopores to detect their three-dimensional structure. More importantly, our system enables data encryption through the use of physical keys. Only the addition of the correct molecular key sequences enables decoding of the data using nanopore sensing, allowing for secure information storage on DNA.

We show the principle of our platform in **Figure 1**. The DNA-HD is made by annealing the 7228 nucleotides (nt) linearized M13mp18 single-stranded DNA (ssDNA) scaffold[27] and complementary oligonucleotides (Figure 1a, Table S1 and Materials and Methods in

the Supporting Information). Oligonucleotides at designed positions are replaced with DNA dumbbell hairpins (introduced in a previous study[9]) or ssDNA overhangs. The DNA is negatively charged so it can be read out using nanopore sensing by applying a potential to drive individual molecules through the sensing volume, which translate the molecular structure information into ionic current signals on the microsecond time scale (Figure 1b). We used ~14 nm nanopores in this study with an example of the Scanning Electron Microscope (SEM) image shown in Figure S1 and characterization details in a former study[9]. Only unfolded translocation events were retained for further analysis as introduced in a previous study.[28] When measured with our ~14 nm diameter nanopores, a group of DNA dumbbells and a streptavidin molecule can create observable downward peaks in the ionic current signal but the ssDNA overhang is too small to be detected.[29] It has been demonstrated that the velocity of the DNA is nearly constant during the translocation,[30] so we can correspond the peak positions in the signal to the actual positions of the attached molecules on the DNA. We employ the dumbbells at designed positions as references (REFs) to indicate the start and end of the reading, measure translocation velocity and determine the directionality of the DNA-HD. High (‘1’) bits are

created by adding biotinylated oligonucleotides that hybridize to the ssDNA overhangs. Monovalent streptavidin binds to the biotin and creates an observable signal at the biotinylated site. Thus, we define data as '0' (without the bound streptavidin) and '1' (with bound streptavidin) between the REFs along the DNA-HD.

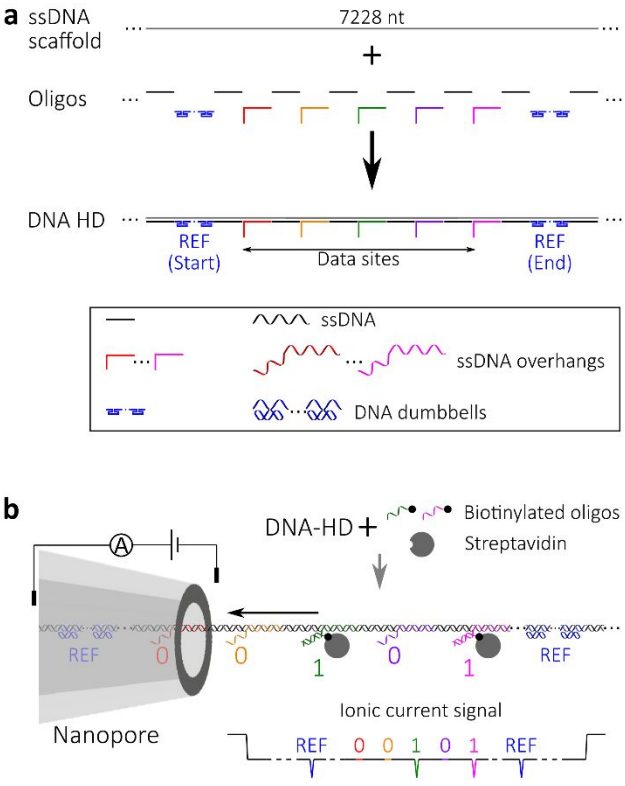

Figure 1. Schematic of the DNA hard drive (DNA-HD). (a) Design of the DNA-HD made from ssDNA scaffold and designed short DNA oligonucleotides to form units for data

storage and operation. The oligonucleotides assemble to the scaffold via sequence specificity and are designed to incorporate structures like ssDNA overhangs and DNA dumbbells at designed locations. The legend is shown in the square. (b) Reading the information on the DNA-HD using a nanopore. Dumbbells are used as fixed references (REFs) to indicate the storage region. Streptavidin and biotin labelled oligonucleotide (complementary to the ssDNA overhang) are added to specifically bind to the ssDNA overhang to generate detectable signals. Here in the example, only oligonucleotides complementary to the green and magenta overhangs are added, so the two sites are read as '1' while the others '0'.

Based on this platform, we now demonstrate that we can write, erase and rewrite data on the DNA-HDs to show the rewritable data storage. To facilitate the proof of concept, the DNA-HD is designed with five sites with ssDNA overhangs between two REF sites (see sequences in Table S2), which we name D1-D5 (Figure 2a). REF sites created by two groups of DNA dumbbells are used to indicate the beginning and end of the data and

determine the direction of DNA-HD according to their predesigned positions. Here, the two REFs are asymmetrically distributed along the DNA. In the current design REFs are only found in one half of the molecule which allows us to easily extract the translocation direction from the signal. Figure 2b shows the schematic workflow of the writing and erasing using D3 as an example. In the initial stage, only a 20 nt overhang partly hybridized with a 10 nt ssDNA blocking strand is on the DNA-HD, which is classified as '0'. The 10 nt provides enough specificity to address all the sites individually. Information is written by hybridizing biotin-labelled ssDNA (Table S3) with the complementary sequence to the overhang ('WRITE'). After the addition of streptavidin, all biotinylated sites present now an observable secondary current peak in nanopore measurements (read as '1'). The biotin-labelled strand includes a free toehold end (black) which is used to remove the streptavidin carrying strand from the carrier by adding a complementary strand (Table S3) using a specific strand displacement reaction. The toehold enables the targeted reset of the bit to '0' ('ERASE'). After erasing of the data, the carrier is ready for a new biotin labelled strand to be added to store information in a new cycle ('REWRITE'). In the above operations, we waited for 1 hour during each procedure (see details in the

Experimental Workflow in the Supporting Information) as a demonstration, which can be considerably shortened by optimizing the experimental parameters.[31,32]

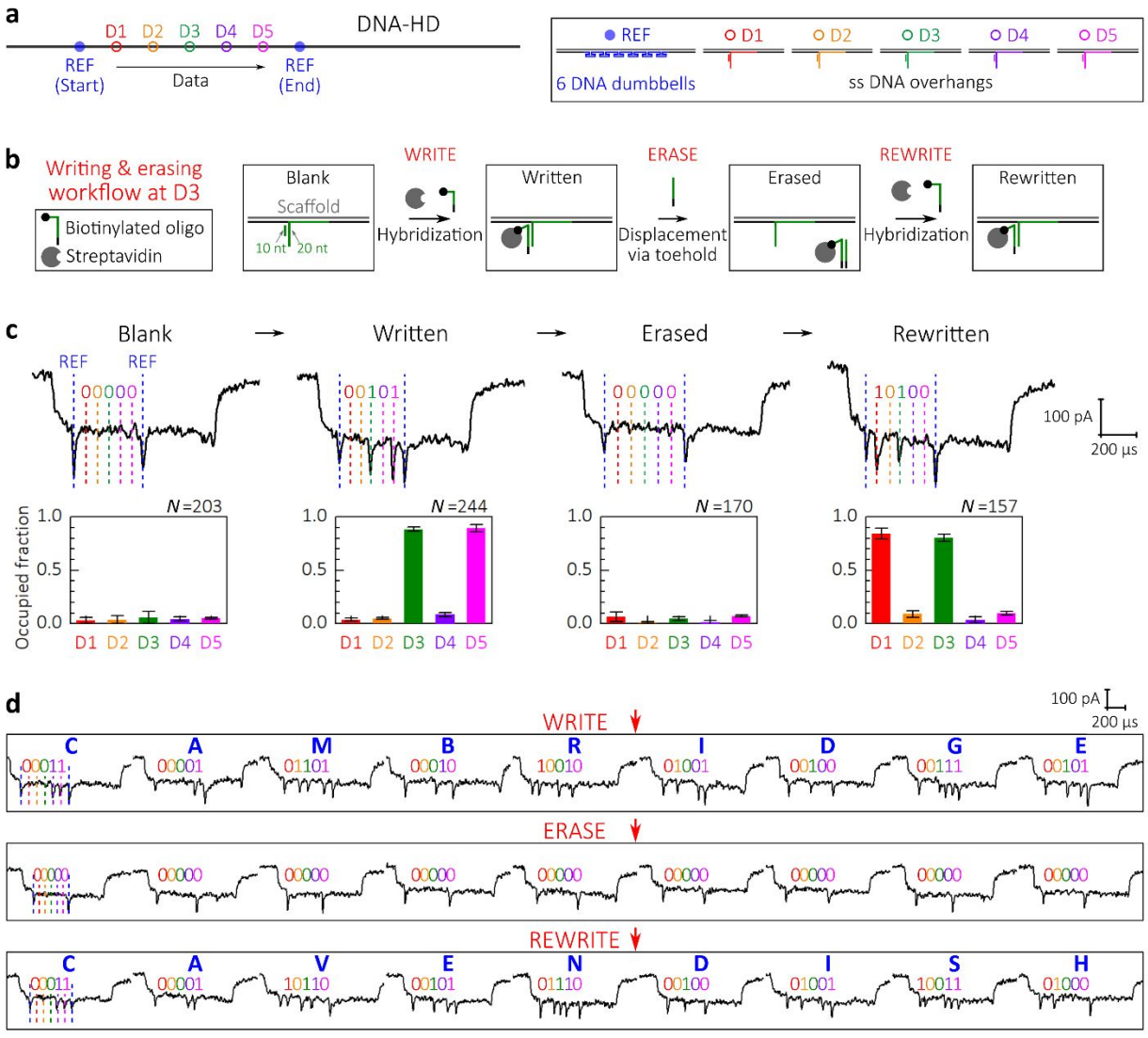

Figure 2. Writing and erasing data on DNA-HDs. (a) Design of a writing system on a 7228 bp long DNA-HD. Two groups of DNA dumbbells (marked as "REF" in blue) are used as references to indicate the start and end of the storage region. Five positions (D1-D5) with ssDNA overhangs are designated (sequences are shown in Table S2). (b) Stages of data writing and erasing on the DNA-HD using D3 as an example. WRITE: A biotin-labelled ssDNA strand is used to write as it binds to the overhang with streptavidin added to cause observable signals in the nanopore reading. ERASE: A designed ssDNA strand erases D3 by removing the bound biotin-streptavidin using a strand displacement reaction. REWRITE: After erasing, new data can be encoded by adding new biotinylated ssDNA strand, completing the writing-erasing-rewriting cycle. (c) Characterization of the performance of the system. '00101' was written and erased, and then '10100' was written. Histograms show the statistics from hundreds of molecules (*N* is the number of molecules) where the occupied fraction means the per cent of the signals read as '1' on the site out of the total molecule number. See the statistics in Table S4. (d) Demonstration of storing letters as 5-bit ASCII on DNA-HDs and reading the data with nanopores. See the statistics in Table S5.

We characterized the performance of our method by writing '00101' (D1-D5), erasing this information and rewriting '10100'. As described above, corresponding oligonucleotides and streptavidin were added in each step and the resulting molecules were analyzed with nanopores (see the Experimental Workflow in the Supporting Information). Figure 2c shows examples of nanopore signals with more events shown in Figure S2. As each streptavidin label caused a secondary current peak in the event, the information was decoded by analyzing the presence or absence of peaks. Our results show a high signal-to-noise (SNR) ratio compared to a recent paper using MoS_2 nanopores for the detection of topological variations on DNA.[33] More importantly, our signals are consistent and the DNA translocation speed is relatively constant, allowing for correct decoding of the information using single molecules. We analyzed hundreds of events and plotted the percentage of detected peaks (named 'occupied fraction') at each site (Figure 2c and Table S4). Initially, all bits had low occupied fractions at the blank state. In the first writing stage, D3 and D5 were written as '1' where the occupied fractions

were close to 80% while they were below 10% for other sites. After the information is erased, occupied fractions dropped again for all sites to below 10%. In the second writing stage, occupied fractions for D1 and D3 were also close to 80% and others were below 10%. These significant changes make it possible to correctly detect the information with high certainty using only a few events. It is noticeable that this error rate is a simple average from multiple single molecules and includes both writing and reading errors which can be fully optimized by improving the experimental parameters and analysis methods. For instance, optimizing the match of nanopore dimensions to the bit size would increase the signal-to-noise ratio. Variation in the translocation velocity can cause errors if the bits are not correctly assigned, thus placing reasonable REFs and controlling DNA velocity by external parameters like electro-osmotic flow, charge or nanopore geometry will help improve the reading accuracy. Another possibility to reduce errors is to encode '0' with smaller structures.[26] Comprehensive data analysis using multiple molecules and machine learning will also significantly improve the error rate.[26,34] After the characterization, we stored words and changed the data using the designed toehold-based rewriting capability (Figure 2d). Nine samples were prepared and measured. Here

we use D1-D5 (corresponding to last 5 bits of ASCII) to encode letters and read them by aggregating the first ten unfolded translocation events for each sample. First, we encoded 'CAMBRIDGE' into the samples with example events and codes shown in Figure 2d (details are shown in Figure S3). Using the same samples, we erased the letters (Figure S4) and wrote 'CAVENDISH' and measured again (Figure S5). The results show we can encode and change data on the DNA-HDs and read the data with nanopores.

The above results show that we can operate on the DNA-HD to achieve rewritable data storage which can also be adapted to DNA computing as well. In the next step, we show our platform can securely store data by encoding information in the DNA nanostructures that cannot be directly read but can be decoded by adding required molecules to reveal the structure information. To this end, we encrypt data on ssDNA overhang sites by applying the requirements of additional complementary sequences labelled with biotin and streptavidin for correct reading. The biotin-labelled complementary oligonucleotide serves as a physical key for data access. Using this idea, we designed sites for data encryption on the DNA-HDs (**Figure 3a**) where we included the predesigned parts for

address (A1-A3) and data (D1-D5) split by the REFs (the REF at the end indicates the direction, see sequences in Tables S6 and S7). The address part is used to identify the order of the data when we need to store data in different domains in a mixture. Here we use DNA dumbbells as unencrypted data sites and ssDNA overhangs as encrypted sites (A3 and D5, Figure 3b), where a blank site (only with ssDNA complementary to the scaffold) means a '0' while a group of dumbbells or the ssDNA overhang a '1'. Figure 3b shows the principle of encryption. For these sites, the address key and data key are the streptavidin and biotin labelled oligonucleotides complementary to the overhangs at A3 and D5 respectively. The data can be only correctly read after the molecular keys are bound to the DNA-HD. One typical example is shown (Figure 3c) where we encoded the word 'SHANNON' (to pay tribute to "the father of information theory") and decoded it with nanopores. We encoded each letter in the data part of a DNA-HD and the order in the address, and then made DNA-HDs and mixed them into a mixture. With both address and data keys presented, we successfully decoded the information with example signals shown in Figure 3d (analysis in Figure S6). However, when the sample was read without the keys, the wrong information appeared (Figure 3e, details in Figure S7). First, the order

of the letters could not be correctly aligned without the correct address. Second, the encrypted letters on the HDs were not correctly retrieved due to the unrevealed D5. Based on this model, the DNA-HDs can be expanded with more address and data sites on each to encode more practical information. It should be noted that we only show the encryption of one bit on the address and one bit on the data to demonstrate the capability. Using our platform, we are able to choose any bit, even all the bits, to be encrypted to allow for ultra-secure data storage. To the best of our knowledge, there is not a straightforward method to decode the information without our physical keys and nanopore sensing platform if all the bits are encrypted. Even more importantly, the information could not even be retrieved with DNA sequencing.

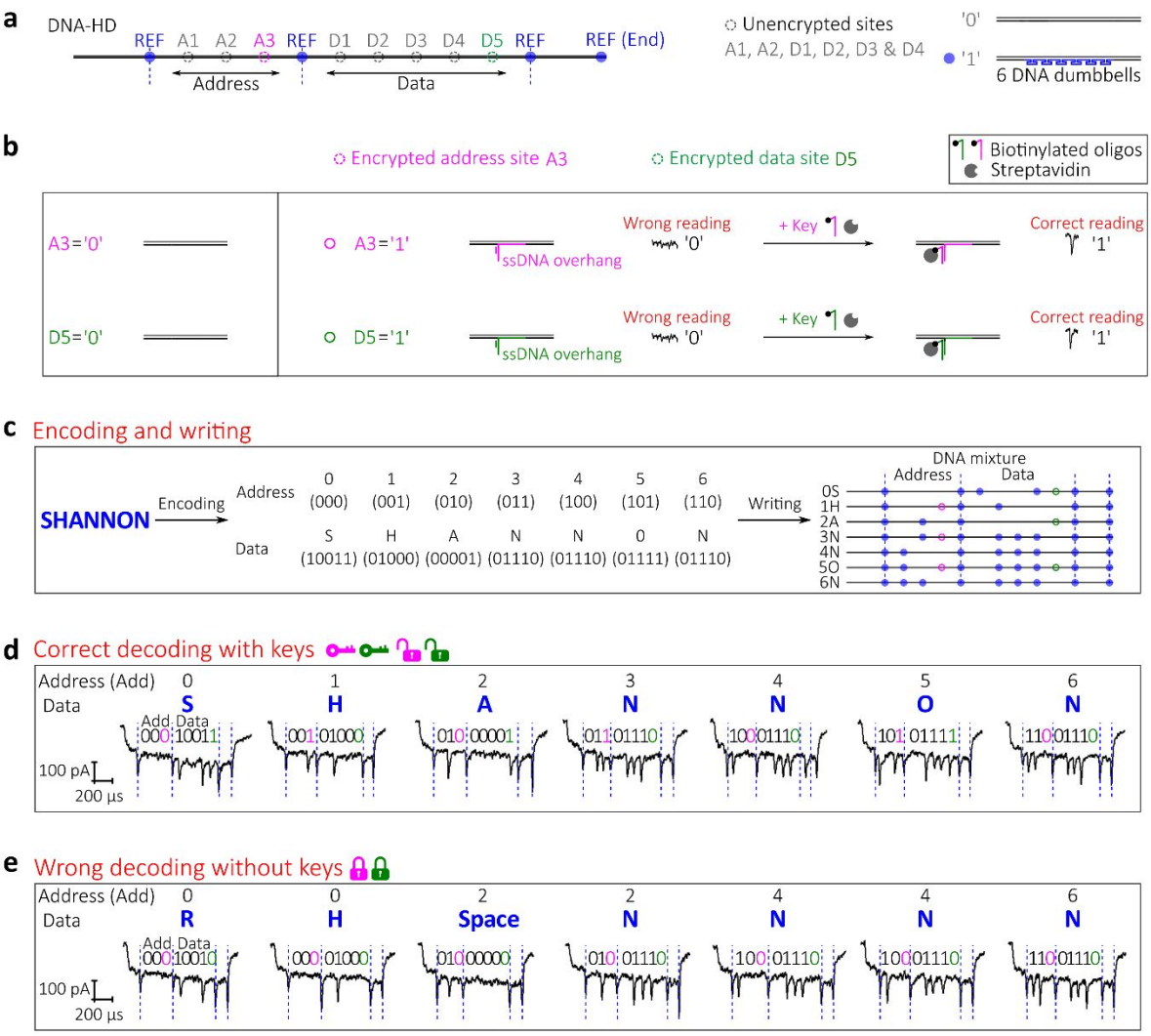

Figure 3. Data encryption on DNA-HDs. (a) Design of the DNA-HD for data encryption with 8 bits used for the example of address and data encoding. Schematics of the unencrypted sites are shown, where blank dsDNA is used to represent '0' and dumbbells '1'. (b) Schematic of the sites for information encryption and the principle of encryption. For these sites, blank dsDNA is used to encode '0' and streptavidin and biotin bound

ssDNA overhang to encode '1'. But the '1' can only be read with the keys (biotin-labelled ssDNA and streptavidin) added. (c) An example of data encryption by encoding "SHANNON" into a mixture of DNA-HDs. (d) Correct decoding the information with the address and data keys. See detailed data analysis in Figures S5. (e) Wrong information decoded without keys. See detailed data analysis in Figures S6.

In this study, we demonstrate the original innovation only by showing encoding words, but our platform can be scaled up by using automated droplet microfluidics in parallel for writing and parallel nanopore measurement for reading.[35] The amplification of these DNA nanostructures is possible if we optimize the design by using closed ssDNA bulges or DNA stem loops[36,37] instead of the open overhangs. Our DNA-HDs hence offer the potential to copy data including random data access by target amplification.[14] Our approach indeed has an inherently lower density compared to data storage in the DNA sequence which is ~10^6 of that in hard drives.[10] In our previous studies, we have demonstrated that our nanopore sensing platform can achieve a resolution of around 100

bp, meaning that we can store one bit per 100 bp and our data density is already ~3 orders of magnitude higher when compared to hard drives.[26] However, the resolution of nanopore for sensing DNA can be significantly improved by using nanopores in thin graphene[38] or MoS2 membranes[32] with thickness below 1 m. The design of DNA nanostructures can also be much smaller and more precise to achieve the storage of one bit of data in tens of base pairs. It should be noted that pushing the limit in data density is only one aspect for molecular data storage as our demonstrated density is already far higher than in conventional hard drives. Synthesis-free and easy writing, convenient and fast reading, potential for integration and adaptability to variable functions are attracting and our platform provides a route towards these directions. Our synthesis-free writing employs only mixing of predesigned building blocks and the nanopore sensing provides a convenient and fast reading, both can be achieved quickly and easily to be integrated into miniature scale. Last but not least, our nanostructure consists of double-stranded DNA (dsDNA) with nicks, so it should be stable enough, at least more stable than complex DNA nanostructures which can be stored for years,[39-41] due to the relative long hybridization length of 38 bp for each short strand.

To conclude, we introduce an approach to store digital information on addressable molecules along dsDNA, which we name DNA-HD. We employ DNA overhangs as sites for adding or removing molecules for dynamic data storage. Using the ssDNA overhangs, we can encrypt data on the DNA-HDs, and decode by adding the physical keys which are the specific biotin labelled complementary oligonucleotides and streptavidin. Without these keys, one is unable to correctly decode the data. Our method with physical keys and nanopore reading is even secured against direct DNA sequencing. Our enzyme-free method has full potential to be automated in combination with microfluidic systems for rapid assembly by mixing. In the future, ever-improving methods under development in DNA nanotechnology will undoubtedly improve both writing capabilities and data security. It is also easier to read secondary structures using nanopores than DNA sequencing, and with our nanopore sensing platform, it only takes microsecond timescale to read data on one DNA-HD suitable for fast reading. In addition, our system can be used for miniature integration to make end-to-end DNA storage devices.[42] We assume our method will become a promising route for DNA data storage as a complementary means to the data

storage in DNA sequence and possibly also for DNA computing due to the flexibility of our platform.

ASSOCIATED CONTENT

Supporting Information. The following files are available free of charge: Materials and Methods, Experimental Workflow, Figures S1 to S7 and Tables S1 to S7.

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Author Contributions

The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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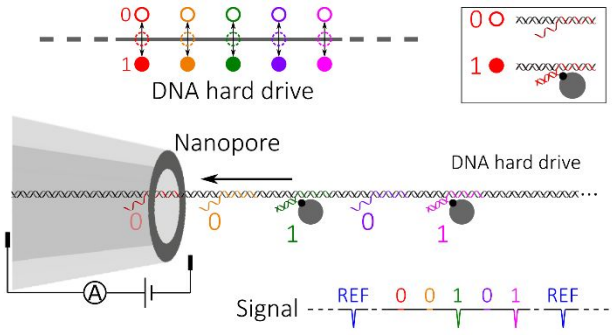
TOC figure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DNA hard drive
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