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# Exchanging and Releasing Information in Synthetic Digital Polymers using a Strand-displacement Strategy

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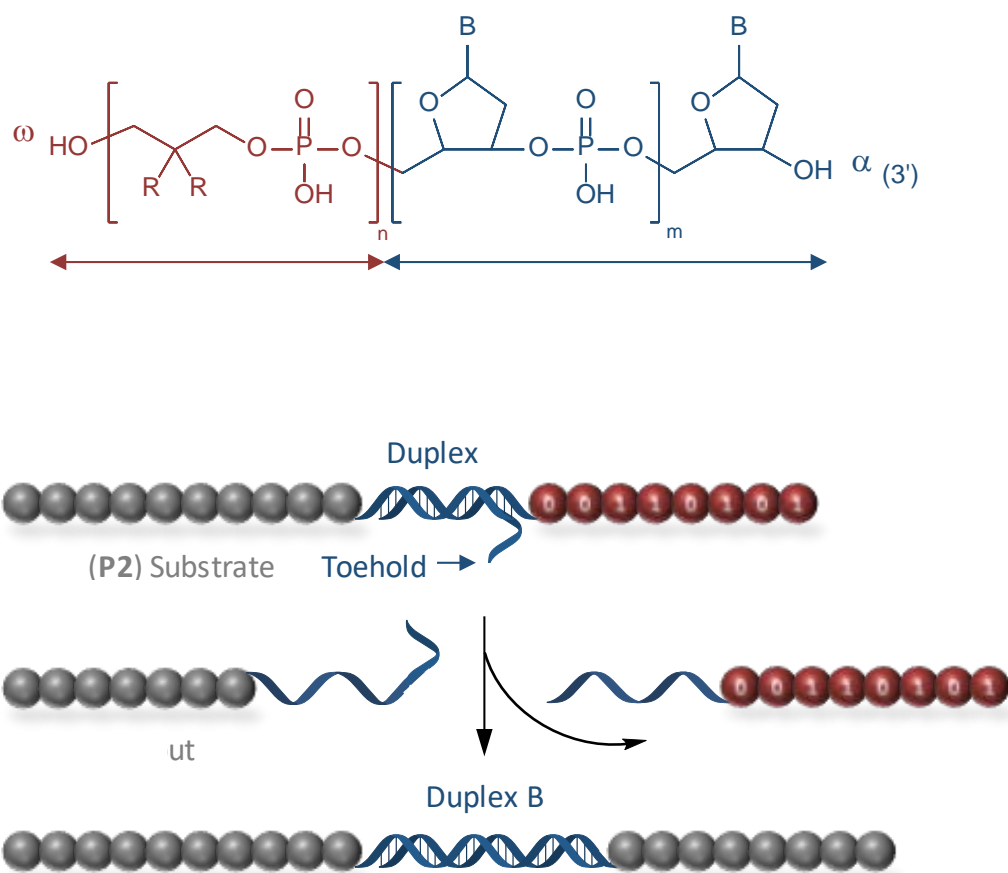
**Abstract.**

Toehold-mediated strand displacement (TMSD) was tested as a tool to edit information in synthetic digital polymers. Uniform DNA-polymer bio-hybrid macromolecules were first synthesized by automated phosphoramidite chemistry and characterized by HPLC, mass spectrometry and polyacrylamide gel electrophoresis (PAGE). These precursors were diblock structures containing a synthetic poly(phosphodiester) (PPDE) segment covalently attached to a single-stranded DNA sequence. Three types of bio-hybrids were prepared herein: a substrate containing an accessible toehold as well as input and output macromolecules. The substrate and the input macromolecules contained non-coded PPDE homopolymers, whereas the output macromolecule contained a digitally-encoded segment. After hybridization of the substrate with the output, incubation in the presence of the input led to efficient TMSD and release of the digital segment. TMSD can therefore be used to erase or rewrite information in self-assembled bio-hybrid superstructures. Furthermore, it was found in this work that the conjugation of DNA single strands to synthetic segments of chosen lengths greatly facilitates the characterization and PAGE visualization of the TMSD process.

Macromolecular data storage consists in storing information in biological or synthetic polymers using monomers as basic information units.<sup>1</sup> Most of the work in this area has been devoted to DNA.<sup>2</sup> Through decades of genomic research, tools for synthesis, amplification, sequencing and self-assembly of nucleic acids are readily available and therefore DNA is a strong option for next generation data storage devices. Yet, DNA is limited by biological constraints. For example, its storage density cannot exceed a theoretical maximum.<sup>3</sup> In comparison, synthetic polymers seem much more versatile because their molecular structure and storage properties can be tuned using a wide variety of building-blocks and reactions.<sup>4-6</sup> Yet, research on synthetic informational polymers has only recently begun and many aspects remain to be studied. For instance, the editing of digital polymers has been barely explored. Some interesting strategies for erasing,<sup>7-8</sup> repairing<sup>9</sup> or rewriting<sup>10</sup> digital polymers have been reported. Yet, these methods all rely on the partial deconstruction - and in some cases reconstruction - of a coded polymer chain. There is also limited work about the spatial organization of synthetic informational macromolecules, although interesting concepts have been reported for organizing libraries of digital polymers in two or three dimensions.<sup>11</sup>

Many limitations listed above could be solved with the use of DNA-polymer bioconjugates (Figure 1a).<sup>12</sup> Indeed, such bio-hybrid macromolecules may combine the advantages of synthetic digital polymers (*e.g.*, high storage density<sup>13</sup>) to those of nucleic acids (*e.g.* programmed self-assembly<sup>14-15</sup>). They can easily be synthesized by phosphoramidite chemistry;<sup>16-17</sup> a method that can be used for the chemical synthesis of DNA and RNA<sup>18</sup> but also for the preparation of synthetic poly(phosphodiester)s (PPDE).<sup>19-21</sup> For instance, in a recent study,<sup>22</sup> uniform DNA-*b*-PPDE block copolymers were prepared by phosphoramidite chemistry and then assembled into linear or non-linear constructs. This simple approach enables the assembly of digitally-encoded PPDEs (*d*PPDE) into programmed information patterns. Yet, the formed superstructures are not dynamic and cannot be easily re-programmed. Here, we introduce a simple strategy

that allows reorganization of digital polymers. It is based on the well-known toehold-mediated strand displacement (TMSD),<sup>23</sup> which is a versatile tool for exchanging DNA strands. This strategy relies on a duplex composed of long and short DNA single-strands, which are called substrate and output, respectively. Having a longer chain-length, the substrate contains a few unpaired nucleobases called toehold. When an input single strand having the exact complementary sequence of the substrate is mixed with the duplex, it first binds to the unpaired toehold and then gradually displaces the output strand until it leads to its release. Initially introduced by Turberfield and coworkers,<sup>24</sup> TMSD has been demonstrated to be a versatile tool for achieving nanomachines<sup>25</sup> and molecular computers.<sup>26</sup> It has also been explored for the synthesis<sup>27-29</sup> or rearrangement of polymer materials.<sup>30-32</sup> In the present study, TMSD was used as a tool to control the information content of DNA-*d*PPDE bio-hybrid assemblies.



**Figure 1.** (a) Molecular structure of the bio-hybrid precursors. (b) Concept studied herein for editing digital assemblies.

**Table 1.** Characterization of the different polymers synthesized in this work.

	Sequence ( $\omega \rightarrow \alpha$ or $5' \rightarrow 3'$ )	Mass [Da] <sup>a</sup>	$m/z_{th}$	$m/z_{exp}$	Yield [%]
<b>P1</b>	00010111110- <i>b</i> -GCATCCCATAACTTACTCCC	7474.2708	1245.2059	1245.2083 <sup>b</sup>	58
<b>P2</b>	[0] <sub>20</sub> - <i>b</i> -GGGAGTAAGTTATGGGATGCGACATGGAGA	12195.7784	1219.0719	1219.0771 <sup>c</sup>	43
<b>P3</b>	[0] <sub>10</sub> -TCTCCATGTCGCATCCCATAACTTACTCCC	10348.5941	1477.9363	1477.9354 <sup>d</sup>	55
<b>ssDNA1</b>	GCATCCCATAACTTACTCCC	5954.0324	1190.2003	1190.1980 <sup>e</sup>	94
<b>ssDNA2</b>	GGGAGTAAGTTATGGGATGCGACATGGAGA	9435.6145	1178.9458	1178.9474 <sup>f</sup>	47
<b>ssDNA3</b>	TCTCCATGTCGCATCCCATAACTTACTCCC	8968.5121	1120.5581	1120.5564 <sup>f</sup>	74

<sup>a</sup>Theoretical monoisotopic mass. Measured at isotopic maximum by negative mode ESI as: <sup>b</sup>[M-6H]<sup>6-</sup>, <sup>c</sup>[M-10H]<sup>10-</sup>, <sup>d</sup>[M-7H]<sup>7-</sup>, <sup>e</sup>[M-5H]<sup>5-</sup>, <sup>f</sup>[M-8H]<sup>8-</sup>.

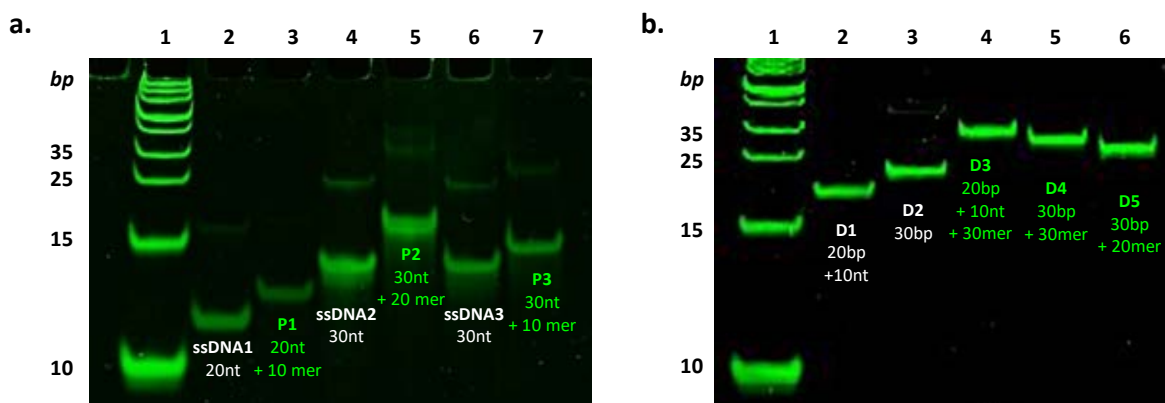
Figure 1b depicts the concept studied herein. Three bio-hybrids **P1-P3** (Table 1, Entries 1-3) were synthesized by automated phosphoramidite chemistry: a substrate, an input and a digital output. All these polymers contain a single-stranded DNA (ssDNA) covalently connected to a synthetic polymer segment. The ssDNA sequences were adapted from the literature to achieve TMSD.<sup>33-34</sup> The substrate **P2** and the input **P3** contain a non-coded homopolymer segment, whereas the output **P1** contains a digitally-encoded copolymer.<sup>35</sup> The chain-length of the synthetic segments was carefully selected in order to allow a visual monitoring of the TMSD process by PAGE (*vide infra*). The three bio-hybrids **P1-P3** were characterized by HPLC (Figure S1) and mass spectrometry (Table 1 and Figures S2-S4). For comparison, the three corresponding ssDNA sequences (Table 1, Entries 4-6) were also synthesized and characterized (Table 1, Figure S1 and Figures S5-S7). For optimal TMSD characterization, it was first essential to select a highly sensitive method for separation and identification of strand-displacement precursors and products. For this purpose, we used a non-denaturing PAGE protocol that enables an

efficient separation of short ssDNA strands (*i.e.*, 20–30 nt long). To limit unwanted intramolecular base-pairing and minimize base-stacking within the ssDNA strands, all samples were heated for 3 minutes at 95°C and snap cooled on ice for 5 minutes. These conditions were first tested for all single-stranded macromolecules (**P1-P3** and **ssDNA1-ssDNA3**) as well as all corresponding double-stranded duplexes (Figure 2). In Figure 2a, it can be seen that the oligonucleotides **ssDNA1-ssDNA3** (lanes 2, 4 and 6) migrate faster than their corresponding DNA-*b*-PPDE conjugates of higher molecular weights **P1-P3** (lanes 3, 5 and 7). These findings are in agreement with previous studies of our group,<sup>36</sup> which indicated that the covalent conjugation of non-natural PPDE segments slows down the electrophoretic mobility of DNA aptamers on agarose gels. When comparing all bare DNA samples, the short output strand **ssDNA1** (20 nt) migrates clearly faster than longer strands (*i.e.* 30 nt). However, **ssDNA2** and **ssDNA3** (*i.e.*, substrate and input for TMSD) have the same chain-length and therefore can barely be separated by PAGE (lanes 4 and 6). Conjugation with PPDE segments of different length permits to solve that issue. For instance, **P3** that contains 10 synthetic units migrates significantly faster than **P2** that contains 20 synthetic units (lanes 7 and 5), although both macromolecules include DNA segments of equal length. Since DNA and PPDE are both polyanions, the net negative charge of the bio-hybrid polymers seems to be the main factor influencing their migration.

**Table 2.** Different duplexes that were studied herein.

	Duplex	Phosphate units <sup>a</sup>
<b>D1</b>	ssDNA1/ssDNA2	20 bp + 10 nt
<b>D2</b>	ssDNA2/ssDNA3	30 bp
<b>D3</b>	P1/P2	10-mer + 20 bp + 10 nt + 20-mer
<b>D4</b>	P2/P3	10-mer + 30 bp + 20-mer
<b>D5</b>	P2/ssDNA3	30 bp + 20-mer

<sup>a</sup> The notations bp, nt and mer denote base pairs, unpaired nucleotides and synthetic PPDE monomers, respectively.



**Figure 2.** Native 20% PAGE obtained for: (a) Single-stranded macromolecules: **ssDNA1** (lane 2), **P1** (lane 3), **ssDNA2** (lane 4), **P2** (lane 5), **ssDNA3** (lane 6) and **P3** (lane 7); (b) Double-stranded duplexes **D1** (lane 2), **D2** (lane 3), **D3** (lane 4), **D4** (lane 5) and **D5** (lane 6). For both **a** and **b**, lane 1 is a commercial DNA ladder (10bp to 300bp). It shall be noted that this ladder is made for double-stranded DNA analysis and therefore shall only be used as a guide for the eyes.

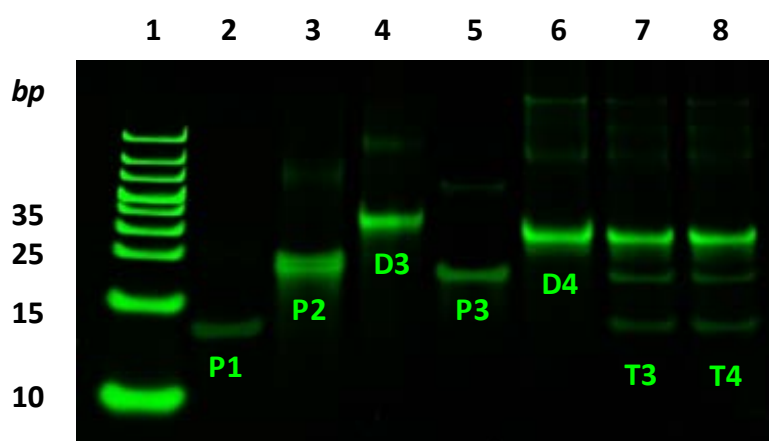
Figure 2b compares the migration profile of different model duplexes **D1-D5** (Table 2). Annealing was performed by mixing individual strands in annealing medium at pH 7, followed by heating at 95 °C and snap cooling on ice. All duplexes were resolved by the PAGE protocol. The migration rate of linear doubled-stranded DNAs through the gel is inversely proportional to the logarithm of their molecular weight and consequently, **D1** migrates slightly faster than **D2** (lanes 2 and 3). For the same reasons as those discussed above for single strands, the duplexes involving bio-hybrid segments **D3-D5** have slower migrations rates (lanes 4 to 6) compared to the pure DNA duplexes **D1** and **D2**. For instance, the comparison of **D4** and **D5** evidences the effect of PPDE conjugation on the migration of duplexes (lanes 5 and 6). Notably, for **D3**, the 30 synthetic units of the PPDE segment and the 10 nucleotides of the toehold influenced synergistically its electrophoretic mobility leading to a migration rate much slower than expected (lane 4).

**Table 3.** Different TMSD experiments studied herein.<sup>a</sup>

	Duplex A	Input	R <sup>b</sup>	Duplex B	Output
<b>T1</b>	D1	ssDNA3	1:1.2	D2	ssDNA1
<b>T2</b>	D1	ssDNA3	1:1.5	D2	ssDNA1
<b>T3</b>	D3	P3	1:1.2	D4	P1
<b>T4</b>	D3	P3	1:1.5	D4	P1
<b>T5</b>	D3	ssDNA3	1:1	D5	P1
<b>T6</b>	D3	ssDNA3	1:1.2	D5	P1

<sup>a</sup> Incubation overnight at RT. <sup>b</sup> Ratio duplex A/input investigated in the experiment.

Based on these model observations, TMSD was afterwards investigated as a mechanism to control information in digital constructs. Table 3 depicts the different TMSD experiments that were conducted in this work. First, the basic mechanism was investigated using bare DNA strands **ssDNA1-ssDNA3** (Figure S8). Two experiments **T1** and **T2** were conducted using different ratios between the starting duplex (Duplex A: substrate/output) and the input strand. In both cases, the incubation with the input clearly leads to the release of the output strand **ssDNA1** (lanes 7 and 8 in Figure S8). However, as discussed above, the duplexes **D1** and **D2** migrate with a close rate (lanes 4 and 6 in Figure S8), making them hard to distinguish from each other.



**Figure 3.** 20% PAGE evaluation of the rewriting mechanism. First, **P1** (lane 2) and **P2** (lane 3) were mixed in equimolar ratio to form **D3** (lane 4). Then, **P3** (lane 5) was incubated with **D3** in

either 1:1.2 (**T3**, lane 7) or 1:1.5 (**T4**, lane 8) molar ratio. Lane 6 shows **D4** for comparison. Lane 1 corresponds to 10–300 bp DNA ladder.

TMSD was then investigated with bio-hybrid macromolecules. For instance, Figure 3 shows PAGE results obtained using **P1**, **P2** and **P3** as output, substrate and input, respectively. Experiments **T3** and **T4** (lanes 7 and 8) clearly show the complete disappearance of starting duplex **D3**, the appearance of the duplex **D4**, the remaining excess of input strand **P3** and the successful release of output strand **P1**. Thus, this simple exchange process can be used as a rewriting mechanism in digital assemblies (*i.e.*, the digital segment of **P1** is replaced by another one in the construct). Similarly, the use of **P1**, **P2** and **ssDNA3** enables a successful erasing mechanism (*i.e.*, the digital segment of **P1** is removed from the construct) as evidenced by the PAGE results shown in Figure S9.

In summary, it was shown in this work that TMSD is a versatile tool to edit synthetic digital polymers. Indeed, the basic principles of rewriting and erasing mechanisms were achieved. Interestingly, DNA was not used in this study as a data storage polymer but rather as a tool allowing organization and exchange of synthetic strands. The studied concept is, of course, not restricted to the minimal models described herein and may be applied to more complex assemblies containing several accessible toeholds. It may also potentially be applied to a wide variety of synthetic PPDEs.<sup>21</sup> Furthermore, sequential mechanisms allowing multiple rewriting procedures may also be envisioned.<sup>37\*</sup>

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