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DNA Logic Gates Integrated on DNA Substrates in Molecular Computing

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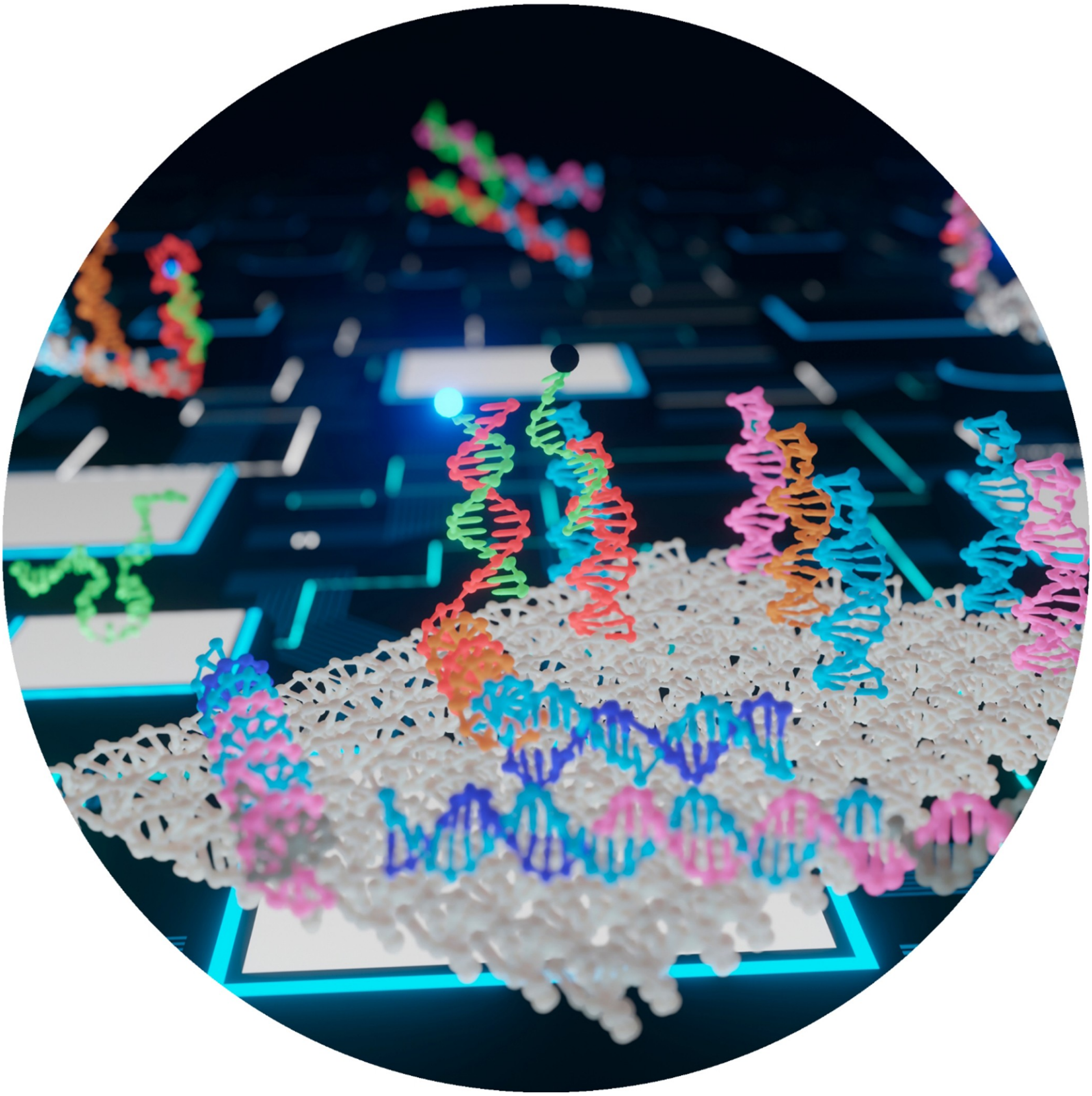

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Due to nucleic acid's programmability, it is possible to realize DNA structures with computing functions, and thus a new generation of molecular computers is evolving to solve biological and medical problems. Pioneered by Milan Stojanovic, Boolean DNA logic gates created the foundation for the development of DNA computers. Similar to electronic computers, the field is evolving towards integrating DNA logic gates and circuits by positioning them on substrates to increase circuit density and minimize gate distance and undesired crosstalk. In this minireview, we summarize recent developments in the integration of DNA logic gates into circuits localized on DNA substrates. This approach of all-DNA integrated circuits (DNA ICs) offers the advantages of biocompatibility, increased circuit response, increased circuit density, reduced unit concentration, facilitated circuit isolation, and facilitated cell uptake. DNA ICs can face similar challenges as their equivalent circuits operating in bulk solution (bulk circuits), and new physical challenges inherent in spatial localization. We discuss possible avenues to overcome these obstacles.

1. Introduction

Modern computers are ubiquitously made of semiconductor materials; however, novel molecular computers can be built from individual molecules acting as computational units. Stojanovic and colleagues reported the first nucleic acid Boolean logic operators[1] and a half adder using RNA-cleaving deoxyribozymes[2] which marked the beginning of an era of DNA computational devices mimicking semiconductor computers.[3–7]

The intrinsic coding feature of nucleic acids and precise base pairing allows for the designing of nucleotide sequences that are capable of completing logic operations[1–9] and that respond to external inputs according to Boolean functions (AND, OR, NOT, etc.).[10,11] More complex logic gates like Feynman and Fredkin, which involve reversible computation,[12–14] adders,[11] subtractors,[15] multipliers,[16] and square roots[17] performing arithmetic logic operations (ALU), solving puzzles, and encrypting information have been reported. Many of these computational building blocks have been designed to work through interactions with enzymes and other proteins, nanoparticles, and quantum dots.[18] These developments suggest that DNA can be programmed to perform similarly to the central processing units (CPU) of electronic computers.

Recent reviews and books on DNA computing focus on the elementary components and toolbox, computing mechanisms, Boolean operators, arithmetic functions and coupling with non-DNA components.[8,18–24] In this minireview, we focus on yet another important trend in developing DNA computers-integrating DNA logic units in communicating chains by tethering them to DNA scaffolds (substrate), named as an all-DNA integrated circuit (DNA IC). The highlighted works are set under the scope of DNA ICs emphasizing different types of DNA substrates and their characteristics including influence in circuit performance, current limitations, and future perspectives

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2. Advantages of DNA-based circuits

Even though the industry of electronic computers pitches their semiconductor transistors in nanometer scale, the distance between gates (also contacted gate pitch, CGP) limits miniaturization as it has reached physical limitations.[24–27] Thus, efforts in further miniaturization inspired exploring new computing materials beyond semiconductor technology including molecules like nucleic acids.

DNA computing might not be at the stage of ultra-fast data processing; however, it circumvents the physical barriers of electronic computers.[21,24] In perspective, a ssDNA gate inherently possesses a gate width of ~1 nm and the lowest CGP currently reported is 5 nm,[28,29] meanwhile the smallest Si-transistor has a 2-fold increase in both gate length and CGP (Figure 1, top). The reduction of CGP is desired because it allows for an increase in gate density. For instance, Intel's 0.1 billion transistors can fit in 1 mm^2,[27] while 40 billion DNA gates could fit in the same area. This is a potential 400 times improvement in transistor density, suggesting the vast room for highly dense DNA nano-circuits. The manufacturing of DNA ICs depends on chemical synthesis and assembly strategies. Although this could be considered expensive and work intensive, the cost per unit should be by far more affordable since billions of DNA computational units can be assembled at once in a small reaction volume.[24] However, the material phase and the interface between DNA ICs and user differs from electronic

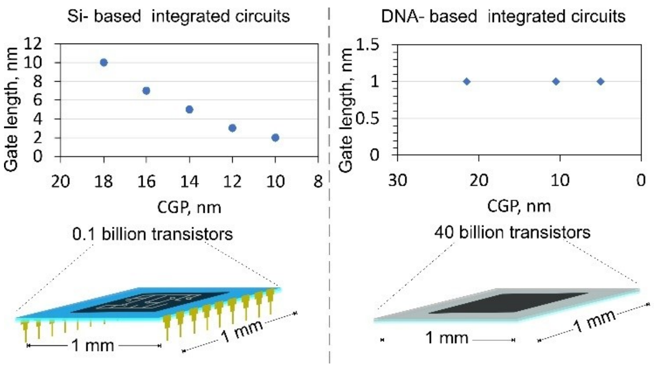

Figure 1. DNA IC in analogy to Si-based IC. Top graphs illustrate the typical CGP values associated with the gate length for Si-based[25] and DNA-based ICs.[28,30,31] Bottom schematics show the number of transistors capable of being integrated on a 1 mm^2 chip.

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computers, making both technologies neither competitive nor compatible with each other.

DNA molecular computing holds the potential for being biocompatible. Thus, it has been envisioned for biological and medical purposes.[18,32] Nonetheless, general purpose DNA molecular computing for digital data storage has been developed showing robust data fidelity after retrieval.[33,34] This feature is expected, considering that DNA is nature's material for data preservation and transmission. In cell-free medium, DNA molecular computers have shown their biosensing capabilities for multiplex diagnostics.[35–38] *In vitro*, DNA logic gates have been proposed for bioimaging, controlled drug delivery, and other theranostic approaches.[39–41] In section 4, we discuss the current stage of DNA ICs *in vitro*, which is one step closer to the overarching goal of autonomous complex DNA computers *in vivo*.

3. Scaling up DNA integrated circuits (mimicking a Si-chip)

A computer made of any material, requires a CPU, which is composed of multiple interconnected logic gates. Most common Boolean logic gates accept one or more inputs and produce ON or OFF output signals, conditioned by a set of rules known as truth table (Figure 2e). Even though DNA logic gates can identify as inputs a myriad of biomolecules (ions,[42] small molecules,[43] nucleic acids,[11] and peptides[41]) as well as non-molecular stimulus (temperature,[44] electromagnetic force,[45] and pH[46]), input and output homogeneity are necessary among each logic gate for their intercommunication. DNA allows for this input/output homogeneity using DNA sequences as inputs and outputs, which interact with the DNA logic gates by formation and/or dissociation of base pairs. As a result, multiple DNA logic gate motifs have been developed, which operate through the association/dissociation of hairpins, four-way junctions (4 J), strand displacement reactions (SDR), RNA/DNA enzyme, and tweezers. (Figure 2).[1,5–8,29,47–51]

3.1. In "bulk" or in "substrate"?

The first DNA circuitries were realized with all gate components diffusing in aqueous solutions (bulk circuits).[1–7,52] Nonetheless, scaling up integrated gates in bulk circuits i) slows response down to hours, ii) requires unique gate sequences to avoid crosstalk, and iii) increases signal leakage and unwanted interactions. Therefore, the design complexity of DNA circuits operating in bulk increases proportionally to the number of communicating gates, since the use of repeating elements must be excluded. This requirement leads to overpopulation of computing components, which increases potential undesired crosstalk and inhibitory interactions.

Table 1 compares the performance of bulk circuits capable of processing ALU. To overcome the mentioned obstacles, common approaches include fuel DNA components and enzymes (to speed up the processes),[53] inhibitory components (to avoid signal leakage),[6,30,52,54] and gate and input libraries (to reduce design complexity).[55] Although, they are useful features, another alternative for addressing the aforementioned problems is anchoring the DNA logic gates to a substrate.[30,56,57] Remarkably, at the cellular level, spatial localization accelerates the interaction between components that are closer to each other and reduces nonspecific crosstalk between them.[58–60] This paradigm has been extrapolated to catalysis, electronic computers and now molecular computing.

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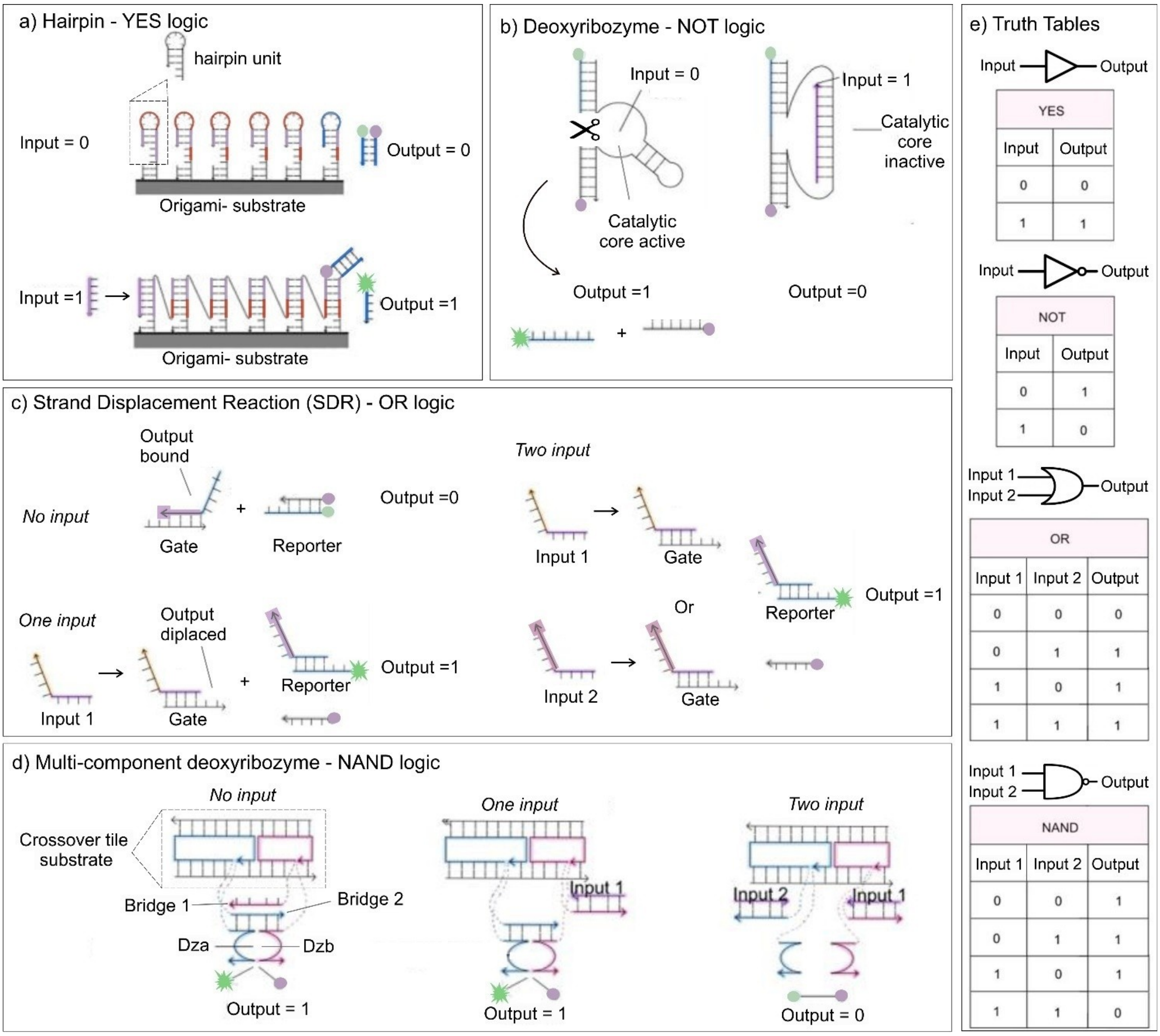

Figure 2. DNA logic gate motifs using fluorescence reporters. High fluorescence is correlated to high output signal (1) and low fluorescence to low output signal (0). Purple dots represent molecular quenchers, green dots-quenched fluorophore, and green stars- fluorescent fluorophores. a) Cascade of 6 hairpin YES gates localized on DNA origami substrate; each YES unit remains as a hairpin in the absence of input, upon input addition (pink ssDNA) the first YES unit opens its stem and communicates with a toehold (red) from a YES unit neighbor, this triggers a chain reaction until a quencher tagged ssDNA is displaced from its fluorophore tagged complement, redrawn from ref. [30]. b) Deoxyribozyme NOT gate in bulk; in the absence of input the catalytic core actively cleaves a substrate into two fragments, one tagged with a fluorophore and the second with a quencher. Input (pink ssDNA) forms a duplex that inhibits the catalytic core from substrate cleavage, redrawn from ref. [1]. c) SDR OR gate in bulk; No input scheme shows Gate holding the Output (pink-blue ssDNA). Adding input 1 (orange-pink) or input 2 (magenta-pink) displaces the bound Output out of the Gate, and Output displaces quencher-tagged ssDNA from its fluorophore-tagged complement, redrawn from ref [54]. d) Multicomponent deoxyribozyme- NAND gate localized on crossover tile substrate; No input scheme shows Bridge 2 holding the deoxyribozyme ssDNA components (Dza and Dzb), which allows catalytic core integrity for substrate cleavage into two fragments. Input 1 binds to Bridge 1, however, Bridge 2 keeps the catalytic core integrity, vice versa if input 2 is added. When Bridge 1 and Bridge 2 are bound to Input 1 and Input 2, the catalytic core falls apart into Dza and Dzb fragments inhibiting substrate cleavage (Two input scheme), redrawn from ref [49]. e) Boolean truth tables dictating the output for all possible inputs for YES, NOT, OR and NAND Boolean logic.

3.2. DNA-substrates for DNA computers, it's all about DNA!

Although multiple materials have been proposed as substrates to spatially localized DNA logic gates (e.g. beads,[20] cell surfaces,[44,61] microarray chips,[22] droplets[62], polymers,[63] and Au films[64]), we narrowed the scope of this minireview to DNA substrates. We consider: i) the potential advantage in bio applications since all-DNA computers allow greater biocompatibility, (ii) the simplicity in circuit layout since DNA logic units can be precisely localized in DNA substrates by hybridization and iii) that DNA substrates are an important player in computer performance. Thus, their current stage and impact in DNA computers development is worth of attention.

DNA is a molecule that can construct scaffolds and structural templates aiding chemical reactions and bio-molecule characterization, the last was demonstrated by Ned

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Table 1. High performance bulk DNA circuitry. ND: Not determined.

Title	Gate motif	Computation				Outcome	Ref
		Max, # gates in cascade	Operator	Processing time, min	Half processing time, min		
Implementing digital computing with DNA-based switching circuits	SDR/ canvas switching circuit	2	YES	< 10	< 3	Fast and scalable computations by routing DNA logic gates in grid	[17]
		3	3-bit input voting	< 12	~3		
		3	Full adder	< 10	< 3		
		2	Square root	ND	< 10		
High-efficiency and integrable DNA arithmetic and logic system based on strand displacement synthesis	Polymerase mediated SDR	2	XOR	~10	~4–6	Developed a ALU using a polymerase mediated strand displacement	[53]
		4	Full adder	~20	~10		
		3	Multiplexer	~12	~5		
		7	1-bit ALU	~20	~5-10		

Seeman.[65,66] The advantages of incorporating DNA substrates over bulk circuitry are: i) ability to closely hold multiple DNA logic processing units,[67] ii) flexibility in spatial arrangement of the integrated units,[30] iii) reusability of functional sequences since localization gives circuit orthogonality,[29,68] iv) isolation of computing elements as one unit,[33] v) facilitate cell uptake and vi) relative stability to nuclease degradation.[69,70]

Paul Rothemund's work lighted a pathway to construct large DNA substrates of different shapes, a technique named DNA origami.[71] At first glance DNA origami offers a large surface area for the anchoring of molecules, thus becoming a widely used substrate.[72,73] The limitation of DNA origami is the yield of the targeted DNA nanostructure, where the moderate (~83–90%) of staple incorporation has been reported.[28,30,68] Thus, incomplete incorporation of structural strands can further compromise gate incorporation and circuit performance. Additionally, scaling up to multi-origami assembly, although possible,[74] might physically hinder intra and inter circuitry processes due to the improper alignment, bending or breaks.[75,76]

One way to circumvent the limitations carried from DNA-origami substrates is the use of small 2D tiles. Our lab explored the use of crossover tiles[49,77] and integrated up to 3 DNA logic gates (Table 2). However, when scaling up in the integration of more logic units, such DNA substrates were prone to bend and misplace the DNA logic units from optimal intercommunication. This was possibly due to the torsional effects and rigidity conferred from the multiple crossover points (Figure 2d and Figure 3d).[49,77] Another proposed alternative is using 3D DNA substrates[10,11,78,79] which we discuss in section 4.

3.3. Localized DNA circuits and their performance

Is it possible to integrate DNA logic gates in long communicating chains like Si-based transistors are integrated on a Si chip? Two research groups theoretically explored the feasibility and performance of cascading DNA hairpin-gates tethered to an origami substrate (Table 2).[67,68] Although gate motif and substrate were in essence similar, the two studies differed in the following aspects: i) addition of untethered components for gate processing and readout; ii) different spatial configurations of gate wiring; iii) probabilistic and kinetic simulations of circuit performance. Dalchau et al. exemplified faster kinetics of DNA ICs predicting completion times of minutes instead of hours as their equivalent bulk circuits. They evaluated 10 elemental YES gates connected in series with a 50% completion time ($t_{1/2}$) of approximately 3.5 min, while shorter times were predicted for cascades with reduced number of gates[68] (Table 2). This work emphasized the need for models that comprehensively reflect the molecular behavior of localized gates to properly evaluate the kinetic behavior of such circuits.

In another approach, Stefanovic and coworkers studied the cascading of molecular spider nanostructures to build DNA ICs.[80] They simulated kinetic behavior and developed an algorithm generating the spatial configuration of the integrated gates on DNA substrates. Their work shows the importance of DNA ICs layout to avoid signal impedance and unwanted interactions.

Experimental works showed that DNA circuit performance can be influenced by the spatial distancing of gates within the DNA substrate. Simmel and coworkers proposed 21.5 nm distance between seesaw (strand displacement-based) gates to avoid signal leakage in the absence of input.[31] Chatterjee et al. separated them by 10.88 nm; however, such 2-fold reduction was allowed by a self-protected motif (hairpin) leading to minimal signal leakage.[30] Lastly, Elezgaray and coworkers distanced each gate by 5 nm with the use of diffusible protector strands[28] or G-quadruplex[29] (Figure 4a), this being the smaller CGP reported. Therefore, far distanced gates have suboptimal communication, while closely distanced gates require additional components to minimize leakage.

The largest wire experimentally tested had 8 YES hairpin gates arranged in a linear cascade with a CGP of 10.88 nm (Figure 3b, right) and produced an output with a $t_{1/2}$ < 10 min (Table 2). In comparison to its bulk counterpart (composed of 9 YES hairpin gates) with a $t_{1/2}$ ~42 min[47], localization on a DNA substrate showed a 4-fold improvement in signal transmission

Table 2. DNA circuits localized on DNA-substrates. ND: Not determined.

Title	DNA substrate	Gate motif	Computation: Max.# gates in cascade	Operator	Processing time, min	Half processing time ($t_{1/2}$), min	Outcome	Ref
Theoretical works								
DNA-based Molecular Architecture with Spatially Localized Components	Origami	Hairpin	3	YES	~480	~180	Spatial localization of DNA hairpins for DNA-based circuit designing	[67]
			2-YES	AND	ND	ND		
			2-AND	Half-Adder	ND	ND		
			4-AND 1-OR	Full adder	ND	ND		
Probabilistic Analysis of Localized DNA Hybridization Circuits	Origami	Hairpin	10	10-YES	ND	~3.5	Development of a method for the probabilistic analysis of localized hybridization circuits.	[68]
			2	OR-AND	~2	~0.5–0.8		
			2	AND-OR	~2	~0.5–0.7		
			3- (AND and OR gates)	Square root (4-bit number)	~1.5-3	~0.7–1		
Experimental works								
Connecting localized DNA strand displacement reactions	Origami 90x60 nm	SDR	2-YES	4th degree fan-out	~4-8	ND	Signal amplification from gates localized on DNA origami	[28]
A spatially localized architecture for fast and modular DNA computing	Origami	Hairpin	8	YES	~40	<10	Increased processing speed of hairpin chain reactions through gate spatial localization.	[30]
			2-AND	3-bit input AND	~20	<6		
			3-AND	6-bit input AND	~40	~12		
			OR-AND	Dual rail XNOR	~15-30	<8		
Robustness of Localized DNA Strand Displacement Cascades	Origami 65x90 nm	SDR	2	YES	ND	~17	Reduced processing time of SDR through gate spatial localization.	[31]
Towards a DNA Nanoprocessor: Reusable Tile-Integrated DNA Circuits	DNA crossover (X) tile	4WJ	AND-2-NOT	NOR	~5	~1	Reusable array of communicating DNA logic gates localized on DNA substrate	[77]
			AND-NOT	INHIBIT	~40	~8		

time. Additionally, the result suggests that 50% of the DNA ICs had released their output in <10 min, accounting for the signal transmission rate of <0.76 nm/min. However, an 8-layered YES wire is a simple system. The increase in the number of connected units on the DNA- substrate in such a fashion expectedly increased the circuit processing time, a similar behavior observed in bulk circuitry.[28,30,31,49,67,68,77]

To speed up signal transmission, approaches such as dual rail input/output (different molecules encode bit-0 and bit-1),[33] and circuit parallelism (independent circuits in simultaneous operation)[30,64] have been suggested. Alternatively, signal can be relayed in different types of arrangement. One example is DNA circuits arranged in a grid pattern, proposed by Wang et al.[17] which reduced the processing time of bulk circuitry (Figure 4c and Table 1). On a DNA substrate, Chatterjee et al. demonstrated the communication of DNA hairpins in a cross-over fashion (Figure 4b). However, using this arrangement for the wiring of more than 8 units was not reported.

In summary, current localized single to multi-layered DNA circuits propagate signal in the range of 1–40 min. This operation time is an improvement on those circuits in bulk which operate at a scale of hours when enzyme-free (Figure 3). DNA substrates allow diverse gate localization and arrangement.[28,30] Additionally, localization enables reducing functional input concentration from 100 nM to 2 nM,[30] allows modular combination of logic gates into various circuits, isolation and storage of computing elements[33] and gate reusability, which reduces the population of computing components. On the other hand, signal dissipation has been observed when connecting their logic gates in series, which could be the result of improper assembly of the units and/or the physical limits of the wiring.

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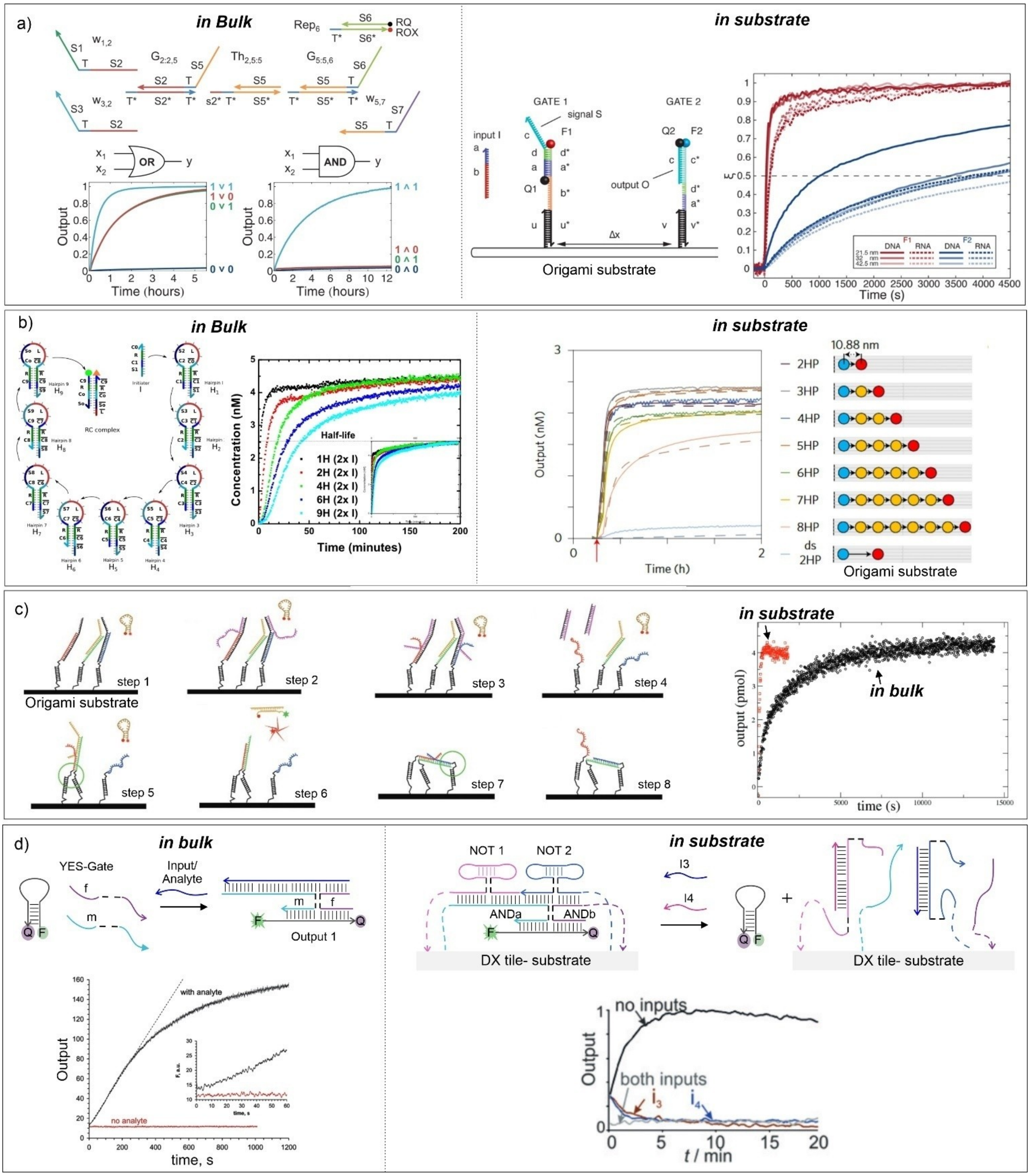

Figure 3. Kinetics comparison of DNA logic gates diffusing in solution (in bulk) vs spatially localized in DNA substrates. Intercommunication of different types of DNA logic gate motif are displayed. Left panels show in bulk: a) Seesaw, b) Hairpin, c) SDR, d) 4 J in bulk, reproduced with permission from [54], [47], [28], [92] respectively. Copyright 2011, The American Association for the Advancement of Science. Copyright 2017, IOP Publishing Ltd and Deutsche Physikalische Gesellschaft. Copyright 2009, Royal Society of Chemistry. Copyright 2013, Elsevier. Right panels show localized a) Seesaw, b) Hairpin (represented by coloured dots), c) SDR, d) 4 J in DNA substrates, reproduced with permission from [31], [30], [28], [77]. Copyright 2014, American Chemical Society. Copyright 2017, Springer Nature. Copyright 2009, Royal Society of Chemistry. Copyright 2016, Wiley-VCH.

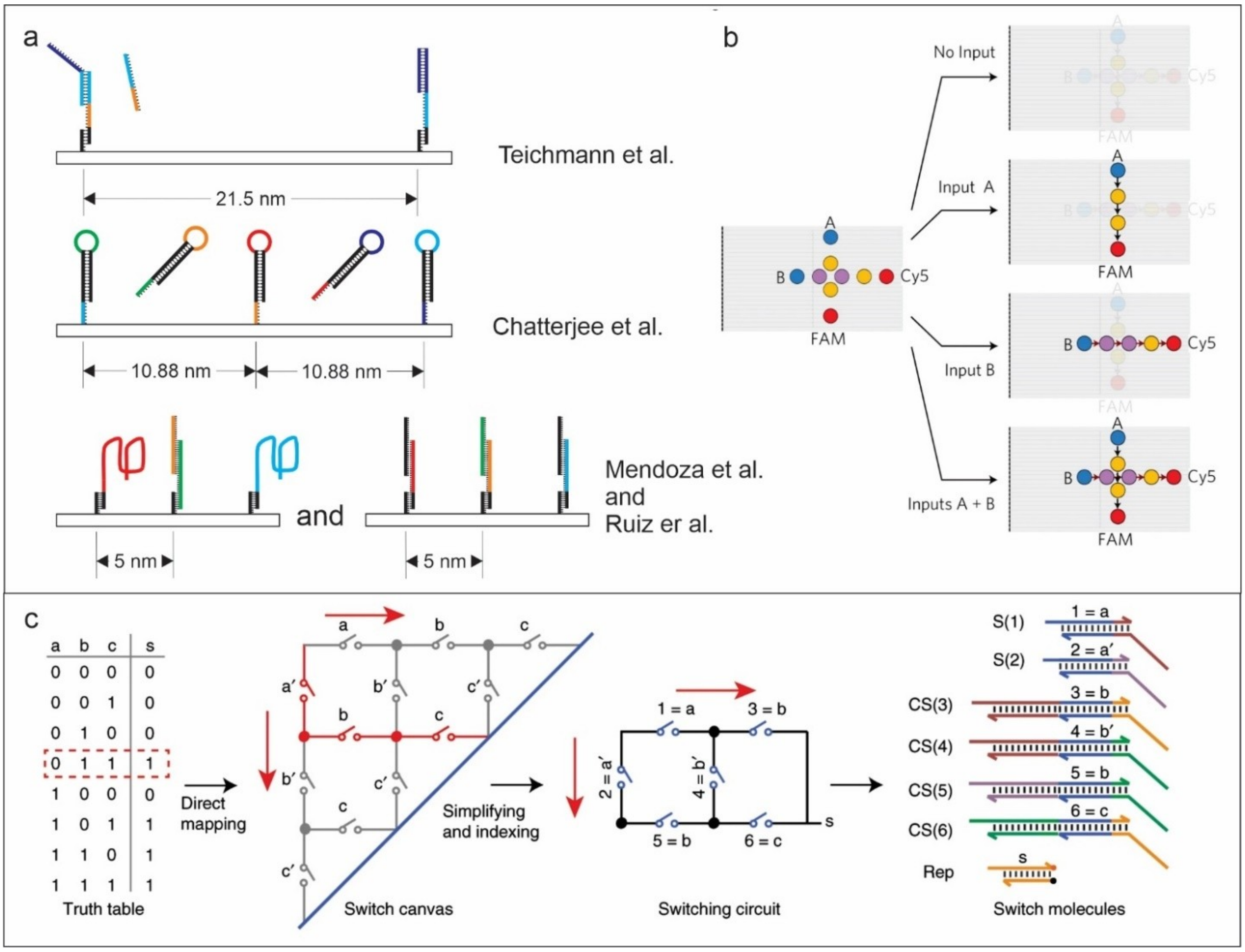

Figure 4. Possible layouts of DNA logic gates on DNA substrates a) Distance between gates on origami substrate, top: seesaw (SDR) middle: hairpins, bottom: G-quadruplex or using of diffusible protector strands, redrawn with permission from [31], [30], [28], [29]. Copyright 2014, American Chemical Society. Copyright 2017, Springer Nature. Copyright 2009, Royal Society of Chemistry. Copyright 2016, American Chemical Society. b) DNA hairpin on origami substrate layout in a communication crossover fashion for signal transmission under different Input combinations. Reproduced with permission from [30]. Copyright 2017, Springer Nature. c) The mapping of signal transmission on DNA-based switching circuits used in the design of bulk circuits in a grid pattern. Reproduced with permission from [17]. Copyright 2020, Fei Wang et al.

4. Biological Applications of DNA ICs

Due to its biocompatibility, DNA logic gates find the following bio-applications: intracellular molecular sensing,[11,42,81,82] gene regulation,[79] triggering cell death,[41] subcellular imaging,[81,83,84] and cell-surface recognition.[85] Various biological analytes (ATP,[11,42,81,83] protons,[11,81,83] metal cations,[11,42,82] miRNA,[79] ssDNA and mRNA,[11,78,81] membrane proteins,[41,85]) have been used as inputs for DNA logic gates. Aptamers, i-motif, MSO sequence, G-quadruplex, DNAzymes, toe hold domains and hairpins were used as sensitive modules of DNA logic gates. However, integration of logic gates as circuits into a single substrate have not been used in an intracellular environment yet. Instead, our literature study has revealed two distinct architectural approaches: i) the integration of DNA logic gates within a single DNA substrate, where gate-to-gate communication occurred, is absent *in vitro* studies;[11,41,42,81–86] and ii) the interaction and regulatory behavior of DNA logic gates localized on separate substrates has been proposed.[79,87]

DNA substrates used *in vitro* studies are usually three-dimensional framework nucleic acids (FNA) (e.g. tetrahedrons, pyramids and cubes),[88,89] which exhibit unique biophysical properties. For instance, after their injection into mice, ssDNA had a half-life of ~15 min, increasing to ~35 min for a tetrahedral DNA.[70] Thus, DNA tetrahedra has a longer half-life than ssDNA in an intracellular environment. In addition, DNA tetrahedrons can be easily taken up by cells.[69]

The ability to produce an easily detectable output signal at low concentrations is important in intracellular sensing of biological compounds. Yang et al. proposed an entropy-driven aggregation of DNA tetrahedron circuits that led to amplification of the output fluorescence signal and improved LOD from nM to fM range.[79] Therefore, further exploration of *in vitro* DNA ICs can be done using these 3D DNA substrates.

5. Perspective and Outlook

Initial DNA circuits executed computational tasks with all the components in bulk. With the scaling up in the chain of intercommunicating units, this approach faced the problems of slow communication rates, non-specific crosstalk, signal dissipation, and overpopulation of computing components. To overcome the problems, the field is evolving to restraining the freedom of the diffusing components. Among a variety of proposed platforms for logic gate localization are DNA substrates.

DNA substrates as computing boards offer the advantage of higher biocompatibility, circuits performing as one computing unit, increased nuclease resistance, increased space for high density circuitry, and ease of DNA ICs isolation. The use of DNA substrates introduces assembly efficiency, an important parameter in the manufacturing of DNA ICs, where the incorporation of structural and logic gate oligonucleotides as the target DNA nanostructure can compromise circuit performance. Although, different 2D and 3D DNA substrate architectures have been proposed, they require *in silico* modeling to minimize and avoid misassembling and communication hindrance. Alternatively, the covalent crosslinking of the DNA substrates during or after assembly[90,91] is an avenue that can be explored to reduce partially assembled structures and disassembly from aging. Another relevant parameter is the distance between intercommunicating gates (CGP) which can be precisely adjusted on DNA substrates. Similarly, CGP is a deterministic factor to Si-based circuits in achieving maximum logic gate density and efficient communication between gates.

DNA ICs are proven to speed up output response and have been able to detect lower concentrations of inputs in comparison to the same ICs performing in bulk. However, the integration of more than 8 DNA logic units on DNA substrates has not been achieved yet, as increasing the number of intercommunicating gates seem to face similar challenges as in bulk. As the number of gates increases in the communicating chain, signal dissipation and slow signal processing rates are unavoidable even with DNA substrates. To mitigate these limitations, signal amplification or transient storage of outputs for later relay to new circuit units are needed-the last showing the relevance of compartmentalization of computing elements for their isolation on DNA substrates.

Alternatively, integration of DNA circuits arranged in grid pattern could significantly speed up localized DNA ICs as in DNA bulk circuit. Although Boolean logic is commonly pursued, non-Boolean circuits, like logic switches could potentially allow for flexibility in gate wiring and increase density of the DNA ICs.

The layout of logic units can affect not only speed but circuit growth and length since localization strings could impede the ability to expand the number of interconnected units. In this regard, the ability to design highly scalable DNA logic circuits is an important aspect to evolve from performing a few numbers of computations to general-purpose computing and automatization.

Circuit designing and analysis software has been used for the planning and wiring of DNA logic circuits *in silico*, which helps to speed up experimental testing and performance troubleshooting. However, such software packages are limited to specific types of DNA gate motifs, limiting their use to less conventional computing nanostructures. Thus, developing a universal software applicable for broader range of DNA gate motifs, substrates, and wiring is needed.

Although delivery of DNA logic units into cells has been reported, no intercommunication of logic gates on DNA substrates as a DNA ICs has been applied in cells up to date. Therefore, we find it important to fill this gap to show the relevance of the integrated DNA circuits to in vivo applications.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Keywords: DNA computing · DNA hybridization · DNA logic circuits · DNA scaffold · Molecular computing

[1] M. N. Stojanovic, T. E. Mitchell, D. Stefanovic, *J. Am. Chem. Soc.* **2002**, *124*, 3555–3561.
[2] M. N. Stojanović, D. Stefanović, *J. Am. Chem. Soc.* **2003**, *125*, 6673–6676.
[3] A. Okamoto, K. Tanaka, I. Saito, *J. Am. Chem. Soc.* **2004**, *126*, 9458–9463.
[4] A. Saghatelian, N. H. Völcker, K. M. Guckian, V. S.-Y. Lin, M. R. Ghadiri, *J. Am. Chem. Soc.* **2003**, *125*, 346–347.
[5] G. Seelig, D. Soloveichik, D. Y. Zhang, E. Winfree, *Science* **2006**, *314*, 1585–1588.
[6] L. Qian, E. Winfree, *J. R. Soc. Interface* **2011**, *8*, 1281–1297.
[7] M. Hagiya, S. Yaegashi1, K. Takahashi, in *Nanotechnol. Sci. Comput.* (Eds.: J. Chen, N. Jonoska, G. Rozenberg), Springer, Berlin, Heidelberg, **2006**, pp. 293–308.
[8] E. Katz, Ed., *DNA- and RNA-Based Computing Systems*, John Wiley & Sons, Weinheim, Germany, **2021**.
[9] L. M. Adleman, *Science* **1994**, *266*, 1021–1024.
[10] R. M. Zadegan, M. D. E. Jepsen, L. L. Hildebrandt, V. Birkedal, J. Kjems, *Small* **2015**, *11*, 1811–1817.
[11] H. Pei, L. Liang, G. Yao, J. Li, Q. Huang, C. Fan, *Angew. Chem. Int. Ed.* **2012**, *51*, 9020–9024.
[12] N. Guz, T. A. Fedotova, B. E. Fratto, O. Schlesinger, L. Alfonta, D. M. Kolpashchikov, E. Katz, *ChemPhysChem* **2016**, *17*, 2247–2255.
[13] A. Sarker, T. Ahmed, S. M. M. Rashid, S. Anwar, L. Jaman, N. Tara, Md. M. Alam, H. Md. H. Babu, *11th IEEE Int. Conf. Bioinforma. Bioeng.*, **2011**, pp. 261–265.
[14] R. Orbach, F. Remacle, R. D. Levine, I. Willner, *Proc. Nat. Acad. Sci.* **2012**, *109*, 21228–21233.
[15] C.-N. Yang, C.-Y. Hsu, Y.-C. Chuang, *Chem. Commun.* **2012**, *48*, 112–114.
[16] G. Wu, N. C. Seeman, *Nat. Comput.* **2006**, *5*, 427–441.
[17] F. Wang, H. Lv, Q. Li, J. Li, X. Zhang, J. Shi, L. Wang, C. Fan, *Nat. Commun.* **2020**, *11*, 121.

[18] C. Kieffer, A. J. Genot, Y. Rondelez, G. Gines, *Adv. Biol.* **2023**, *7*, 2200203.
[19] J. Liu, S. Liu, C. Zou, S. Xu, C. Zhou, *IEEE Trans. NanoBioscience* **2023**, *22*, 245–258.
[20] M. N. Stojanovic, D. Stefanovic, S. Rudchenko, *Acc. Chem. Res.* **2014**, *47*, 1845–1852.
[21] C. Zhang, L. Ge, Y. Zhuang, Z. Shen, Z. Zhong, Z. Zhang, X. You, *Sci. China Inf. Sci.* **2018**, *62*, 61301.
[22] Z. Zhang, C. Fan, L. He, *Curr. Nanosci.* **2005**, *1*, 89–93.
[23] D. Fan, J. Wang, E. Wang, S. Dong, *Adv. Sci.* **2020**, *7*, 2001766.
[24] R. E. Polak, A. J. Keung, *Curr. Opin. Biotechnol.* **2023**, *81*, 102940.
[25] H. H. Radamson, X. He, Q. Zhang, J. Liu, H. Cui, J. Xiang, Z. Kong, W. Xiong, J. Li, J. Gao, H. Yang, S. Gu, X. Zhao, Y. Du, J. Yu, G. Wang, *Micromachines* **2019**, *10*, 293.
[26] S. Ye, L. Liu, Y. Ma, Y. Wang, *Silicon* **2023**, *15*, 2467–2478.
[27] N. Vidhya, V. Seethalakshmi, S. Suganyadevi, in *Quantum Comput. Shift Bits Qubits* (Eds.: R. Pandey, N. Srivastava, N. K. Singh, K. Tyagi, Springer Nature, Singapore, **2023**, pp. 131–150.
[28] I. M. Ruiz, J.-M. Arbona, A. Lad, O. Mendoza, J.-P. Aimé, J. Elezgaray, *Nanoscale* **2015**, *7*, 12970–12978.
[29] O. Mendoza, J.-L. Mergny, J.-P. Aimé, J. Elezgaray, *Nano Lett.* **2016**, *16*, 624–628.
[30] G. Chatterjee, N. Dalchau, R. A. Muscat, A. Phillips, G. Seelig, *Nat. Nanotechnol.* **2017**, *12*, 920–927.
[31] M. Teichmann, E. Kopperger, F. C. Simmel, *ACS Nano* **2014**, *8*, 8487–8496.
[32] M. You, G. Zhu, T. Chen, M. J. Donovan, W. Tan, *J. Am. Chem. Soc.* **2015**, *137*, 667–674.
[33] H. Lv, N. Xie, M. Li, M. Dong, C. Sun, Q. Zhang, L. Zhao, J. Li, X. Zuo, H. Chen, F. Wang, C. Fan, *Nature* **2023**, 1–9.
[34] A. Doricchi, C. M. Platnich, A. Gimpel, F. Horn, M. Earle, G. Lanzavecchia, A. L. Cortajarena, L. M. Liz-Marzán, N. Liu, R. Heckel, R. N. Grass, R. Krahne, U. F. Keyser, D. Garoli, *ACS Nano* **2022**, *16*, 17552–17571.
[35] S. Mirzayi, H. Ravan, S. Soltanian, *Int. J. Biol. Macromol.* **2022**, *207*, 1005–1010.
[36] S. Zhao, L. Yu, S. Yang, X. Tang, K. Chang, M. Chen, *Nanoscale Horiz.* **2021**, *6*, 298–310.
[37] A. Romieu, *Org. Biomol. Chem.* **2015**, *13*, 1294–1306.
[38] P. G. Moerman, R. Schulman, *Nat. Nanotechnol.* **2020**, *15*, 626–627.
[39] C. Wang, Z. Zhang, Y. Qiu, Y. Bao, Q. Song, B. Zou, *Anal. Chem.* **2024**, *96*, 756–765.
[40] J. Chen, S. Fu, C. Zhang, H. Liu, X. Su, *Small* **2022**, *18*, 2108008.
[41] D. Wang, S. Li, Z. Zhao, X. Zhang, W. Tan, *Angew. Chem. Int. Ed.* **2021**, *60*, 15816–15820.
[42] H. Wang, J. Zheng, Y. Sun, T. Li, *Biosens. Bioelectron.* **2018**, *117*, 729–735.
[43] J. Elbaz, B. Shlyahovsky, D. Li, I. Willner, *ChemBioChem* **2008**, *9*, 232–239.
[44] Y. Li, J. Deng, Z. Han, C. Liu, F. Tian, R. Xu, D. Han, S. Zhang, J. Sun, *J. Am. Chem. Soc.* **2021**, *143*, 1290–1295.
[45] K. Wu, C. Qi, Z. Zhu, C. Wang, B. Song, C. Chang, *J. Phys. Chem. Lett.* **2020**, *11*, 7002–7008.
[46] M. Qi, P. Shi, X. Zhang, S. Cui, Y. Liu, S. Zhou, Q. Zhang, *RSC Adv.* **2023**, *13*, 9864–9870.
[47] H. Bui, S. Garg, V. Miao, T. Song, R. Mokhtar, J. Reif, *New J. Phys.* **2017**, *19*, 015006.
[48] M. N. Stojanovic, P. de Prada, D. W. Landry, *ChemBioChem* **2001**, *2*, 411–415.
[49] T. A. Molden, M. C. Grillo, D. M. Kolpashchikov, *Chem. Eur. J.* **2021**, *27*, 2421–2426.
[50] L. Ge, W. Wang, X. Sun, T. Hou, F. Li, *Anal. Chem.* **2016**, *88*, 9691–9698.
[51] D. Bai, J. Zhang, Y. Zhang, H. Yu, L. Zhang, X. Han, K. Lv, L. Wang, W. Luo, Y. Wu, X. Zhou, W. Wang, T. Feng, G. Xie, *Small* **2023**, 2307421.
[52] M. N. Stojanovic, D. Stefanovic, *Nat. Biotechnol.* **2003**, *21*, 1069–1074.
[53] H. Su, J. Xu, Q. Wang, F. Wang, X. Zhou, *Nat. Commun.* **2019**, *10*, 5390.
[54] L. Qian, E. Winfree, *Science* **2011**, *332*, 1196–1201.
[55] J. Elbaz, O. Lioubashevski, F. Wang, F. Remacle, R. D. Levine, I. Willner, *Nat. Nanotechnol.* **2010**, *5*, 417–422.
[56] H. Chandran, N. Gopalkrishnan, A. Phillips, J. Reif, in *DNA Comput. Mol. Program.* (Eds.: L. Cardelli, W. Shih), Springer Berlin Heidelberg, Berlin, Heidelberg, **2011**, pp. 64–83.
[57] K. Lund, A. J. Manzo, N. Dabby, N. Michelotti, A. Johnson-Buck, J. Nangreave, S. Taylor, R. Pei, M. N. Stojanovic, N. G. Walter, E. Winfree, H. Yan, *Nature* **2010**, *465*, 206–210.
[58] R. P. Bhattacharyya, A. Reményi, B. J. Yeh, W. A. Lim, *Annu. Rev. Biochem.* **2006**, *75*, 655–680.
[59] J. D. Scott, T. Pawson, *Science* **2009**, *326*, 1220–1224.
[60] B. N. Kholodenko, *Nat. Rev. Mol. Cell Biol.* **2006**, *7*, 165–176.
[61] Y. Zhang, W. Chen, Y. Fang, X. Zhang, Y. Liu, H. Ju, *J. Am. Chem. Soc.* **2021**, *143*, 15233–15242.
[62] H. Yasuga, K. Inoue, R. Kawano, M. Takinoue, T. Osaki, K. Kamiya, N. Miki, S. Takeuchi, *PLoS One* **2017**, *12*, e0180876.
[63] W. Engelen, S. P. W. Wijnands, M. Merkx, *J. Am. Chem. Soc.* **2018**, *140*, 9758–9767.
[64] S. Piranej, A. Bazrafshan, K. Salaita, *Nat. Nanotechnol.* **2022**, *17*, 514–523.
[65] N. C. Seeman, *Biochemistry* **2003**, *42*, 7259–7269.
[66] N. C. Seeman, *Nature* **2003**, *421*, 427–431.
[67] R. A. Muscat, K. Strauss, L. Ceze, G. Seelig, *Int. Symposium on Computer Architecture* **2013**, *3*, 177–188.
[68] N. Dalchau, H. Chandran, N. Gopalkrishnan, A. Phillips, J. Reif, *ACS Synth. Biol.* **2015**, *4*, 898–913.
[69] A. S. Walsh, H. Yin, C. M. Erben, M. J. A. Wood, A. J. Turberfield, *ACS Nano* **2011**, *5*, 5427–5432.
[70] T. Zhang, T. Tian, R. Zhou, S. Li, W. Ma, Y. Zhang, N. Liu, S. Shi, Q. Li, X. Xie, Y. Ge, M. Liu, Q. Zhang, S. Lin, X. Cai, Y. Lin, *Nat. Protoc.* **2020**, *15*, 2728–2757.
[71] P. W. K. Rothemund, *Nature* **2006**, *440*, 297–302.
[72] P. Zhan, A. Peil, Q. Jiang, D. Wang, S. Mousavi, Q. Xiong, Q. Shen, Y. Shang, B. Ding, C. Lin, Y. Ke, N. Liu, *Chem. Rev.* **2023**, *123*, 3976–4050.
[73] F. Hong, F. Zhang, Y. Liu, H. Yan, *Chem. Rev.* **2017**, *117*, 12584–12640.
[74] A. Rajendran, M. Endo, Y. Katsuda, K. Hidaka, H. Sugiyama, *ACS Nano* **2011**, *5*, 665–671.
[75] Y. Ke, S. M. Douglas, M. Liu, J. Sharma, A. Cheng, A. Leung, Y. Liu, W. M. Shih, H. Yan, *J. Am. Chem. Soc.* **2009**, *131*, 15903–15908.
[76] S. Woo, P. W. K. Rothemund, *Nat. Chem.* **2011**, *3*, 620–627.
[77] Y. V. Gerasimova, D. M. Kolpashchikov, *Angew. Chem. Int. Ed.* **2016**, *55*, 10244–10247.
[78] L. Song, M. Xiao, W. Lai, L. Li, Y. Wan, H. Pei, *Chin. J. Chem.* **2021**, *39*, 947–953.
[79] S. Yang, J. Luo, L. Zhang, L. Feng, Y. He, X. Gao, S. Xie, M. Gao, D. Luo, K. Chang, M. Chen, *Adv. Sci.* **2023**, *10*, 2301814.
[80] D. Mo, M. R. Lakin, D. Stefanovic, *BioSystems* **2016**, *146*, 10–25.
[81] H. Wang, P. Peng, Q. Wang, Y. Du, Z. Tian, T. Li, *Angew. Chem. Int. Ed. Engl.* **2020**, *59*, 6099–6107.
[82] D. Zhu, J. Huang, Y. Xia, S. Su, X. Zuo, Q. Li, L. Wang, *Chemosensors* **2023**, *11*, 358.
[83] Y. Du, P. Peng, T. Li, *ACS Nano* **2019**, *13*, 5778–5784.
[84] B. Groves, Y.-J. Chen, C. Zurla, S. Pochekailov, J. L. Kirschman, P. J. Santangelo, G. Seelig, *Nat. Nanotechnol.* **2016**, *11*, 287–294.
[85] R. Peng, X. Zheng, Y. Lyu, L. Xu, X. Zhang, G. Ke, Q. Liu, C. You, S. Huan, W. Tan, *J. Am. Chem. Soc.* **2018**, *140*, 9793–9796.
[86] Z. Zhou, N. Lin, Y. Ouyang, S. Liu, Y. Zhang, I. Willner, *J. Am. Chem. Soc.* **2023**, *145*, 12617–12629.
[87] Y. Amir, E. Ben-Ishay, D. Levner, S. Ittah, A. Abu-Horowitz, I. Bachelet, *Nat. Nanotechnol.* **2014**, *9*, 353–357.
[88] R. P. Goodman, I. A. T. Schaap, C. F. Tardin, C. M. Erben, R. M. Berry, C. F. Schmidt, A. J. Turberfield, *Science* **2005**, *310*, 1661–1665.
[89] C. Tian, X. Li, Z. Liu, W. Jiang, G. Wang, C. Mao, *Angew. Chem. Int. Ed.* **2014**, *53*, 8041–8044.
[90] N. Weizenmann, G. Scheidgen-Kleyboldt, J. Ye, C. B. Krause, D. Kauert, S. Helmi, C. Rouillon, R. Seidel, *Nanoscale* **2021**, *13*, 17556–17565.
[91] T. Gerling, M. Kube, B. Kick, H. Dietz, *Sci. Adv.* **2018**, *4*, eaau1157.
[92] Y. V. Gerasimova, D. M. Kolpashchikov, *Biosens. Bioelectron.* **2013**, *41*, 386–390.

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