DNA Nanotechnology in Oligonucleotide Drug Delivery Systems: Prospects for Bio-nanorobots in Cancer Treatment

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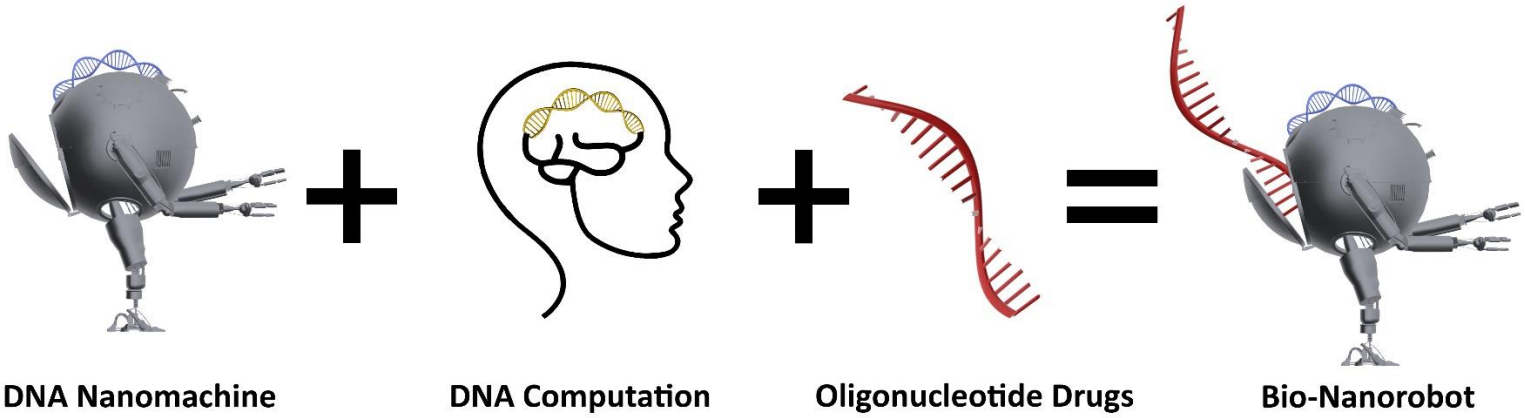
Abstract

DNA-based nanomaterials have shown great potential in numerous applications, thanks to their unique properties including DNA's various molecular interactions, programmability, and versatility with biological modules. Meanwhile, the DNA origami platforms have shown promise in the creation of drug carriers. This technique has paved the way for the production of nanomachines with outstanding performance. Moreover, DNA's encoding capability and its massive parallelism help us to manipulate it for DNA computation. The DNA nanotechnology method holds potential, particularly for oligonucleotide therapeutics that enable precision medicine for cancers.

Here, we explore the potential of DNA nanotechnology in this context, focusing on the DNA origami method and our efforts to streamline its production process. We then delve into studies demonstrating the application of DNA nanotechnology in delivering oligonucleotide drugs for tumor targeting. Following this, we examine DNA-based dynamic nanodevices that can be activated through molecular binding, environmental stimuli, and external field manipulation. Subsequently, we investigate the role of DNA computation in the production of logic gates, DNA circuits, data storage, and machine learning. Finally, we envision the future development of 'bio-nanorobots' based on DNA, enabled by advancements in DNA computation. We propose that combining DNA computation with DNA nanomachines could facilitate the realization of this vision, distinguishing it from conventional drug delivery systems.

Keywords: DNA nanotechnology, DNA origami, Oligonucleotide drug delivery, Nanorobots, DNA computation

Graphical Abstract



Introduction

DNA nanotechnology is experiencing rapid growth and utilizes deoxyribonucleic acid (DNA) as a primary tool to create artificial structures suitable for numerous diverse applications. Manipulating DNA, which is a well-defined structure with a diameter of about 2 nanometers and a helical pitch of about 3.4 nanometers per turn, outside of its biological environment to develop, investigate, and apply DNA-based structures has resulted in the possibility of taking advantage of DNA's chemical and physical properties at the nanoscale. This has led to remarkable progress in controlling molecular self-assembly and revolutionizing nanoscience, and nanotechnology. The diverse use of DNA in biological areas, such as disease prevention, antitumor drug delivery, diagnosis, bioimaging, biosensing, tissue regeneration, and inflammation inhibition, has been fueled by advances in DNA nanotechnology[1], [2], [3], [4].

The programmability and predictability of DNA are more outstanding than other natural or synthetic materials[1]. In addition, DNA nanotechnology uses a variety of intermolecular interactions to manipulate the formation and attributes of DNA nanostructures (Fig.1), including:

1. Hydrogen bonding Watson–Crick base-pairing to design and assemble synthetic DNA strands into complex structures with high precision and specificity. One of the effective bottom-up fabrication techniques based on this type of interaction, in DNA nanotechnology, named DNA origami, which is raster-filling a pattern by folding a long ssDNA called 'scaffold' by binding with some short strands, called 'staple', via baseparing to make the whole pattern stable. This well-defined methodology, which can be assisted by computational designation, has provided the possibility of precisely knitting various patterns and structures from one-dimensional (1D) to three-dimensional (3D) or even hierarchical assemblies[2], [5], [6], [7]. The DNA tile-based method, which is also based on hydrogen bonding interaction derived from immobile branched DNA junctions[8], is the other approach in synthesizing DNA nanostructures[9], [10] and can also be used in crystals and periodic assemblies with characterization and programming of designed arbitrary shapes and geometries[1], [11], [12], [13].
2. Stacking interactions between adjacent base pairs in the DNA helix, mediated by van der Waals forces, contributes to the overall stability and rigidity of the double helix structure and thus stabilizes the assembly of DNA nanostructures, which is effectively applied in designing dynamic nanodevices[14], [15].

3. Electrostatic interactions between the negatively charged phosphate backbone of the DNA and multivalent cations that can be taken advantage of in conformational changes in DNA nano-assemblies[14], [16]
4. Hydrophobic interactions due to the basic section of the DNA can offer advantages in constructing DNA-based biomaterials, such as stabilizing DNA, enhancing interaction with other nanomaterials, adjustability, and enabling the construction of higher-order self-assembly structures all of which can be improved by increasing the hydrophobicity of the DNA as well, to synthesize DNA-based amphiphilic block copolymer[16].
5. Metal coordination through phosphate groups or nitrogenous bases of the DNA, which can be applied in DNA-metal nanomaterials by assisting baseparing hybridization[17], [18].
6. Conjugation with the other modules via modifying the functional groups at the ends of DNA strands that can be assisted by other types of interactions such as basepairing or hydrophobicity to form micelles, tubes, and vesicles or other various architectures which also protect against enzymatic degradation and improves cell uptake[16], [19], [20], [21]. Capability of conjugation with other bio modules can also help in shaping the macroscopic morphology, from small hydrogels to large ones[22]

Moreover, DNA's thermodynamic stability arises from the hydrogen bonding between the complementary base pairs and the stacking interactions between adjacent base pairs. This stability allows DNA to maintain its double helix structure under a wide range of conditions and protects genetic information[23].

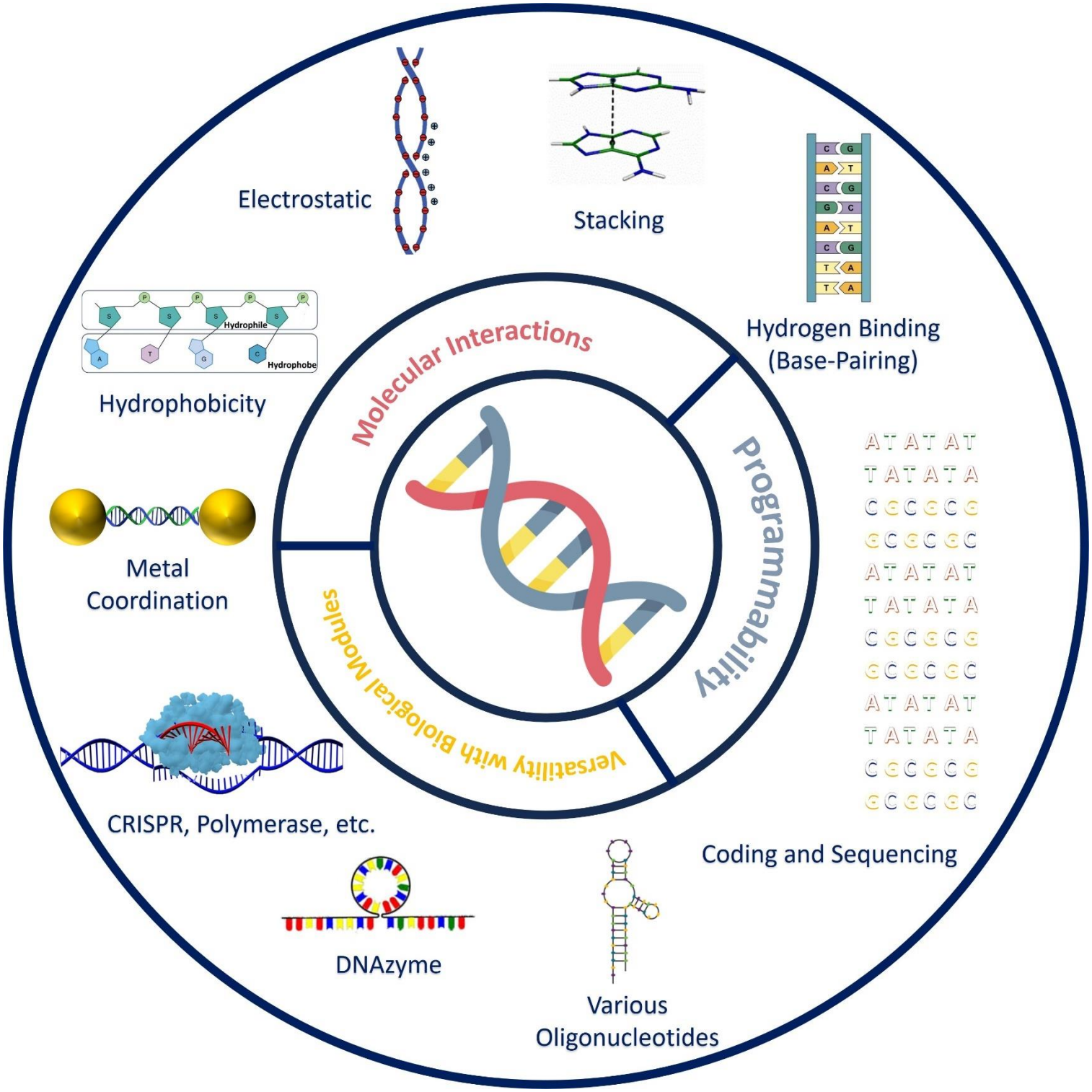


Figure 1- DNA nanotechnology presents numerous benefits, opportunities, and capabilities surpassing those of other soft materials in drug delivery. This is attributed to its diverse molecular interactions, adaptability with biological components, and its distinct programmability, setting it apart from other molecules.

Numerous studies have established that DNA possesses many characteristics of soft materials, including its deformability and responsiveness to various stimuli such as biomarker binding, pH, and electric actuation. Furthermore, DNA exhibits remarkable versatility, being compatible with not only a diverse range of molecules such as some other types of polymers or amphiphiles but also with biological and functional components like proteins and enzymes.

However, what truly sets DNA and oligonucleotides apart from other materials is that they are fully encoded molecules capable of storing vast amounts of genetic information (Fig.1). They are highly programmable, and researchers have developed numerous efficient methods for editing, modifying, and synthesizing encoded oligonucleotides. These unique properties make DNA an easily controlled molecule that can be manipulated at the molecular, nano, or even micrometer scale, with high-throughput and low-cost techniques[12] that make it play an essential role in various areas of study.

The various applications of DNA-based nanomaterials have demonstrated significant potential, thanks to the many highlighted benefits. In this discussion, after we address the production considerations associated with utilizing the DNA origami method as the most noteworthy platform, we explore the potential of DNA nanotechnology as a viable strategy for achieving treatment with oligonucleotide drug delivery. By reviewing DNA nanotechnology in dynamic nanodevices and looking through DNA computation, we envision the opportunities and possibilities that could be realized through the application of DNA-based technologies in the delivery of nucleic acid drugs in precision medicine of cancer therapy.

Production aspects of DNA origami-based platforms

The DNA's ability to form base pairs has brought numerous benefits to DNA nanotechnology. It is the most widely utilized molecular interaction in research studies and the design of DNA drug delivery systems. There are two methods based on this interaction, namely 'DNA tile-based' and 'DNA origami'. Due to some limitations, including being error-prone and time-consuming of the former method, and also restrictions in controlling the stoichiometry and purity of DNA components in the designation of desired structures[24], the latter method has garnered greater attention which is introduced by Rothemund in 2006[6].

DNA origami structures have gained interest as carriers for drug delivery due to their biodegradability, low cytotoxicity, and ability to load diverse therapeutic molecules. They can enter cells and cross biological barriers, accumulating in solid tumors and showing potential for treating kidney injuries. Ligand molecules can be incorporated for active targeting. DNA origami structures offer customizable and precise drug delivery with minimal off-target effects. Smart carriers capable of sensing environmental stimuli and adapting their structures and properties are needed for effective drug delivery. DNA origami structures have been engineered as nanorobots with logic-gating capabilities, facilitating targeted delivery of drug molecules to specific cell types via conditional exposure. They can be unfolded selectively in tumor-associated blood vessels, promoting thrombosis and inhibiting tumor growth. DNA origami structures offer higher loading capacity and complex patterning compared to smaller DNA objects, making them advantageous for drug delivery. Challenges include stability in biological environments and immunogenicity, but these can be mitigated through rational engineering in well-folded DNA nanostructures. Furthermore, in fields such as cancer immunotherapy, DNA architectures with increased immunogenicity can be utilized as adjuvants.[2], [25].

Comprehensive frameworks for DNA origami technology methodologies have been established, covering origami design, synthesis, functionalization, and characterization. Different software tools like caDNAno, ATHENA, and Adenita have been employed for the design of DNA origami structures. Among these, Adenita stands out as it is the sole software capable of accommodating other biomolecules such as drug molecules, lipids, or proteins alongside DNA in the design process[2]. Recently, a versatile computer-aided iterative design pipeline for DNA assemblies has been reported. This framework enables the efficient construction of large, complex assemblies with precise control over geometry, mechanical

properties, and dynamics. The combination of top-down automation and bottom-up control will boost the design potential of intricate DNA assemblies, while also reducing the time required for their creation[26].

The selection of scaffold for DNA origami, such as the m13mp18 viral genome, depends on size and complexity, and staple strands can be purchased as oligonucleotides or produced by oligo synthesizers. DNA origami yield depends on cation concentration, typically using buffers with varying Mg^{2+} concentrations. After that, self-assembly is done through thermal annealing, with duration based on complexity. Here, denaturing polyacrylamide gel electrophoresis (PAGE) purification is used for dynamic structures. Stepwise assembly may be employed for integrating materials or creating hierarchical structures. Achieving high-yield complex DNA nanostructures at a constant temperature would be valuable. DNA origami structures assemble through base stacking interactions between terminal bases, promoting geometric matching and surface contact for shape complementarity in hierarchical assemblies. To ensure reproducibility in DNA origami assembly, several key aspects need to be considered. Optimization of annealing procedures and cationic strength is crucial for target product yield and avoiding by-products. Purification methods, such as size-exclusion chromatography, ultracentrifugation, gel or filter purification, and polyethylene glycol (PEG) precipitation, should be chosen based on factors like yield, duration, and residual contaminants. Purifying and concentrating intact origami nanostructures to high concentrations of greater-scale structures, which typically involves synthesizing a high quantity of staple strands, is still the significant barrier to their practical use in biological applications. The stabilization of DNA origami structures is dependent on the suitable storage temperature and cationic strength, with options including thermal stability, photo-cross-linking-assisted stability, lyophilization, and the use of block copolymers for long-term storage. The success of the self-assembly process is determined by utilizing ensemble methods, such as UV-visible spectroscopy, gel electrophoresis, fluorescence spectroscopy along with circular dichroism. Although providing information about the overall characteristics of the assembled molecules through these techniques, individual details may remain unresolved. DNA origami structures provide a surface for the precise positioning of chemical species at nanometer distances. Each nucleobase can be chemically modified, allowing the precise placement of molecules along the helical axis with a spacing of 0.34 nm. Methods such as single-molecule fluorescence microscopy, transmission electron microscopy (TEM), and atomic force microscopy (AFM) have been applied to examine and characterize DNA origami formations experimentally[2][24]. It appears that purification remains a significant challenge that hampers the practicality and productivity of DNA origami production methods. This challenge arises from various factors, including the actual efficiency of oligo synthesizer instruments achievable in practice and the need for strict controls to be implemented throughout the process, such as preventing moisture from entering the system. One possible approach to address these challenges could be changing the basis of production methods beyond chemical synthesis to include biological synthesis, similar to the high-throughput and adequately efficient procedure that naturally occurs in living cells. For instance, the exploration of enzymatic tools or methods inspired by the process through which the DNA primase (Fig.2C), a ribonucleic acid (RNA) polymerase type, catalyzes the synthesis of short RNA molecules during DNA replication such that the enzymatic process can be utilized for staples and short strands production.

Studies showed that mass production of DNA origami even inside the cell or on a micrometer scale could be high-throughput and low-cost[12], [27], [28]. This research has shown that bacteriophages can efficiently generate single-stranded DNA of various lengths and sequences, enabling scalable and cost-effective production. The bacteriophage-based process involves the creation of precursor DNA containing target sequences interspersed with self-excising

'cassettes', each containing two DNAzymes cleaved by Zn^{2+}[27]. While there are still some challenges in vivo production of DNA origami structures, some studies have shown that it is not impossible to achieve. A flexible method for creating precise tile-based nanostructures by folding individual long single-stranded RNAs (ssRNAs) has been devised in which each nanostructure comprises a single ssRNA molecule, and they can be cloned, expressed, and self-folded in E. coli[29]. Hence, developing a method for in vivo or intracellular production could replace chemically synthesizing single-stranded staples or scaffolds, it might eliminate the need for purification or at least reduce the purification effort due to the potentially high production yield. Adopting a "molecular manufacturing" approach could be possible to adopt an approach like manufacturing processes. Just as workers operate within factories to manufacture products, artificial cells (as factories) could serve as suitable environments for enzymes, proteins, DNAzymes, or other functional biomolecules (as workers) to execute necessary catalytic functions on oligonucleotides, producing DNA staples and scaffolds, or even facilitating self-assembly of DNA structures within these artificial cells (Fig.2). Using artificial cells in place of biological ones could aid in removing unwanted elements and regulating processes through the manipulation and design of cells to perform desired functions. Additionally, employing CRISPR/Cas9 with encoded guide RNA may assist in trimming a lengthy DNA strand containing all target sequences of staples (Fig.2A), while polymerases (Fig.2B) and primases (Fig.2C) may be applied to replicate longer and shorter RNA strands, respectively, and the reverse transcriptases (Fig.2D) produce the DNA strands, afterward. Next, the generated strands undergo self-assembly in the subsequent stage, either within the artificial cell or in vitro. Given the practicality of this efficient and less errorprone method, the purification step will be minimized in the DNA origami fabrication procedure, helping streamline the whole process of DNA production and reducing the cost of production in terms of both time and energy.

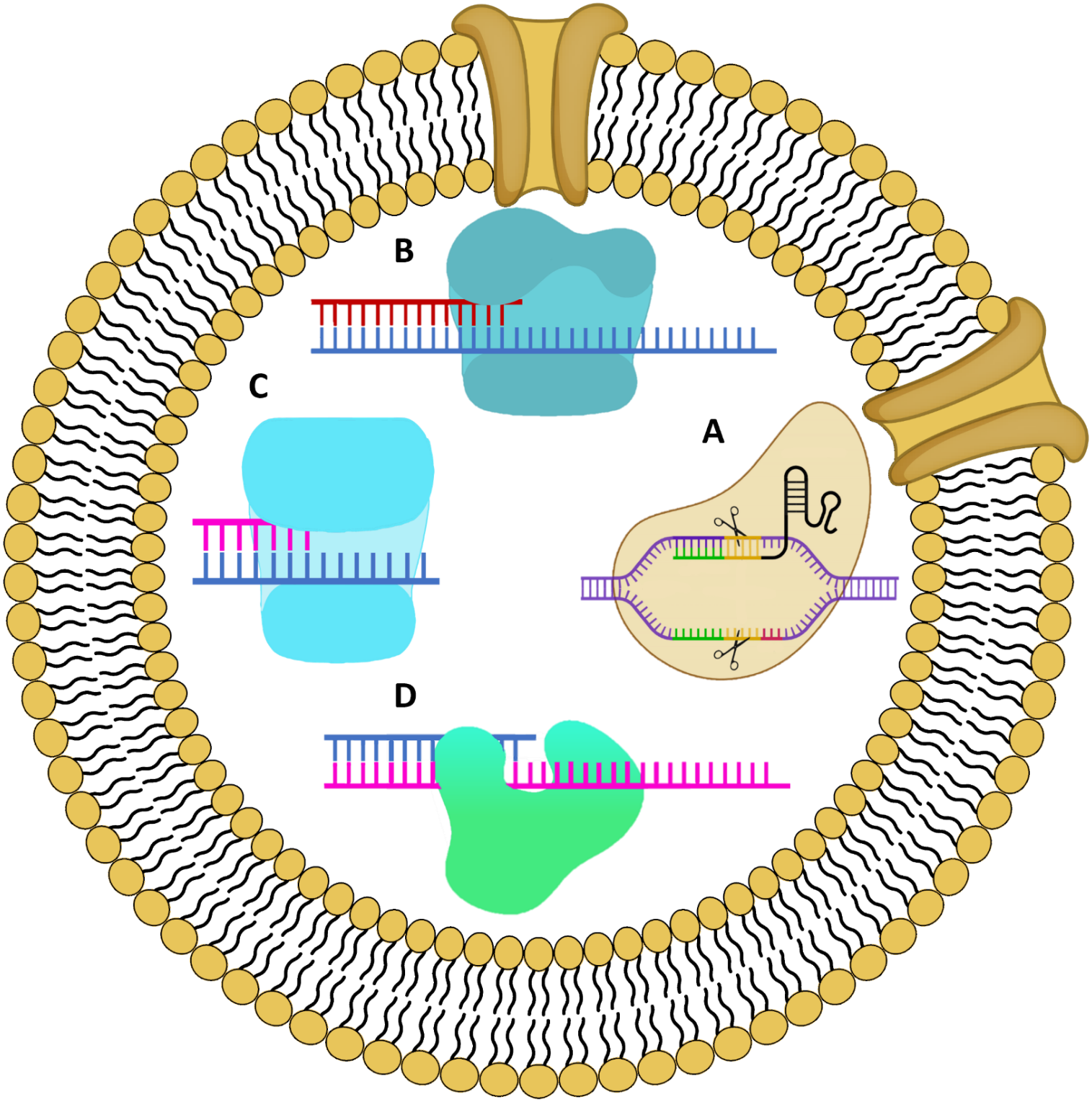


Figure 2- Prospect method for the production of DNA origami in artificial cells using A) CRISPR/CAS9 to trim a dsDNA containing all staples and scaffolds sequences, B) polymerases, C) primases to replicate long and short strands derived from trimming, and D) reverse transcriptases to produce final DNA strands used in DNA origami fabrication.

Based on recent findings, the obstacles faced in progressing DNA nanostructure-based anti-tumor agent delivery may be primarily related to technical hurdles, regulatory considerations, and ethical implications, rather than financial limitations. This indicates that there is potential for this approach to be a lucrative and effective therapeutic method [30]. Authorization approval

of an oligonucleotide anti-cancer therapeutic utilizing the DNA origami platform for delivery could potentially lead to a significant market boost for these therapeutic approaches.

DNA nanotechnology approach in oligonucleotide drug delivery in cancer treatment

Cancer remains among the top contributors to fatality globally. The year 2018 witnessed 9.5 million deaths caused by cancer among 18.1 million new instances and it is projected that there will be 29.5 million newly diagnosed cancer cases, by 2040, and 16.4 million cancer-related deaths annually[31], [32]. Thus, more efficient measures should be tackled in prevention, diagnosis, and treatment. Oligonucleotide drugs are among possible different cancer therapies which refer to chains of polynucleic acids that can be either modified or unmodified. These chains contain different functional groups depending on their purpose and origin. Small interfering RNA (siRNA), antisense oligonucleotides (ASO), microRNA (miRNA), aptamer, and CpG oligonucleotides (CpG ODNs) are some of the most studied oligonucleotide therapeutics in recent research. Their mechanism of action could be modifying RNA, altering protein expression, gene silencing, or stimulating the production of desired proteins[33]. A number of oligonucleotide medications have recently received approval from the U.S. Food and Drug Administration (FDA) for the treatment of rare diseases[34]. While there are currently no FDA-approved oligonucleotide anti-cancer agents, there is optimism surrounding multiple research studies that are investigating the use of oligonucleotide therapeutics in cancer treatment and oncology. Cancer treatment faces challenges with restricted efficiency and harmful side effects of conventional drugs. Other challenges include tumor heterogeneity and high mutation rates. Oligonucleotide therapeutics are being recognized as a potentially effective class of drugs, with the ability to target proteins and non-coding RNAs that were previously considered inaccessible. which is not possible with chemotherapy medication. They also offer potential solutions for precision anti-cancer medicine. Clinical trials are underway, with the expectation of reaching the market soon[35], [36].

Although oligonucleotide drugs can bind to definite targets very specifically, there are many challenges regarding their delivery including the large size, negative charges, problems in passing through the barriers, such as the plasma membrane, plasma proteins, immune systems, blood–brain barrier and the possibility of renal clearance or nuclease degradation[37]. Modification in different parts of the molecule or bioconjugation with GalNAc[38] or bottlebrush polymers[39], for instance, are some strategies to relieve these challenges[37][37]. Loading on nanocarriers, however, would be more effective in enhancing delivery to tumor targets. Making complex with polymers in polyplexes, such as polyethylene glycol- poly(L-lysine) (PEG-PLL) as a hydrophilic-cationic copolymer not only can confer the negative charge problem of high payloads and prevent nuclease degradation by providing steric hindrance but also can help in various cell uptake and crossing some physiological barriers[41][40]. Lipoplexes which are the other vesicle types for delivering condensed oligonucleotides can be composed of ionizable lipids to relieve the toxicity of vehicles and prevent inflammation due to positive charge and make a more efficient endosomal escape, as well. By adding special lipids, selective organ delivery could be also possible [43][41]. Nonetheless, liposomes and polymeric nanoparticles encounter drawbacks such as instability, quick drug release, restricted drug loading capacity, poor biocompatibility, and unsuitability for large-scale manufacturing[42]. In terms of nucleic acid delivery, the possibility of partial degradation of the drug after complexation with the cationic polymers, colloidal instability of lipoplexes and polyplexes in the extracellular compartment of the body, reticuloendothelial system (RES), extravasation, possible problems regarding biodistribution or organ and cellular targeting are

among the challenges in designing a suitable delivery system[43]. There are also some toxicologic concerns with current organic and inorganic medical nanoparticles[44].

In comparison to inorganic particles or lipid-based systems, which are conventional nanoscale drug carriers, DNA-based nanostructures could be more efficient candidates due to the high programmability of their sequence, the predictability of their interactions, their great stability in physiological environments, precisely controllability in design, delivery, release, and targeting, structural diversity, their capacity for high payloads, high biocompatibility, low cytotoxicity, biodegradability, and high bioavailability. They can be easily taken up by many types of cells by various methods such as the endocytosis pathway without transfection. They have the ability to effectively cross biological barriers and specifically target drug sites, minimizing non-specific effects. While DNA nanostructures are versatile with different therapeutic strategies, including immunotherapy, chemotherapy, phototherapy, and gene therapy, high levels of flexibility and modifiability, which are the intrinsic properties of the DNA, not only can help in the multi-functionalizing of the DNA nanomaterials with various ligands through different ways such as conjugation, encapsulation, intercalation, and loading, but also provide the feasible integration of diverse functional modules into DNA-based nanocarriers, allowing for real-time tracking, conditional responding, and controlled release. All of these mentioned benefits along with the inherent compatibility of DNA nanostructures with therapeutic oligonucleotide drugs [4], [24], [25], [45], [46], [47], make DNA nanotechnology a significant suitable approach to developing smart-stimuli responsive drug delivery systems that are not only responsive to physiological stimuli but also can behave logically. These nucleotide based nanostructures make the production of logical nanomachines and intelligent nanorobots feasible.

Some studies have utilized DNA structures for oligonucleotide delivery or gene therapy. Examining the uptake of tubule-like tile-based DNA nanostructures, carrying siRNA, folic acid, and fluorescent dyes, by GFP-expressing HeLa cells[48], a nanoplatform constructed from branched DNA developed to simultaneously deliver gene editing (sgRNA/Cas9, which targets DNA in the nucleus) and gene silencing (antisense, targeting mRNA in the cytoplasm) components integrating with an active targeting aptamer for the purpose of targeting the tumor-associated gene PLK1[49], are among some examples of oligo drugs delivery with DNA platforms. The initial demonstration of DNA origami as a framework for co-delivering a chemotherapy drug and a meticulously arranged gene to target multidrug-resistant tumors (specifically MCF-7R) both in laboratory settings and within living organisms involved a DNA nanokite decorated with various functional elements for targeted delivery and regulated release, including the MUC1 aptamer[50]. DNA tetrahedrons, distinguished by their precise shape and size control, outstanding biocompatibility, numerous sites for targeted decoration, stability, and straightforward synthesis, exhibit considerable potential for molecular diagnosis and targeted drug delivery, including nucleic acids[51], such as self-assembled triangular DNA nanoparticle hybridized with mTOR single-stranded siRNA resulting in high transfection efficiency into NCI-H292 cells[52] or a double-bundle DNA tetrahedron integrated with antisense oligonucleotides knockdowning proto-oncogene c-raf without transfection agents[53]. Crosslinked DNA chains with hydrophilic polymeric networks in DNA hydrogel-based stimuli-responsive delivery systems are also widely investigated[54]. DNA origami-based nanobricks of rectangular and tubular shapes with varied dimensions were utilized to deliver siRNA to anti-apoptotic protein Bcl2, in vitro and in vivo[55]. It was demonstrated that the addition of a cationic polymer layer on a DNA nanoclew (NC) core, carrying Cas12a/CRISPR RNA (crRNA) ribonucleoprotein (RNP), not only made the nanomaterial surface, negatively charged under a physiological pH but also reverted to positive charge under an acidic environment and showed significantly reducing the expression of PCSK9 which resulted in

cholesterol regulation[56]. A tubular DNA origami nanodevice loaded by siRNA and doxorubicin was developed with additional components including tumor-penetrating ligands and stimuli-responsive DNA locks. These locks, containing disulfide bonds, kept the nanodevices closed until they encounter reducing agents like glutathione in tumor cells, triggering their opening and releasing the siRNA cargo. The nanodevice also incorporates cell-penetrating peptides to enhance cellular uptake. By targeting the key proteins involved in tumor progression, in vitro and in vivo experiments demonstrated effective gene silencing and combined cancer therapy at the tumor site[57].

Despite the utilization of diverse delivery methods for nucleic acid drugs in cancer therapy[58], there remains significant potential for exploring DNA nanotechnology in this context. We envision that leveraging the adaptability of both the drug and its carrier could facilitate the development of bio-nanorobots endowed with dynamic capabilities and computational potency, enabling precise content delivery and release. Before exploring our prospects for a bio-nanorobot transporting oligo drugs, we conduct a review of advancements in DNA dynamic nanodevices and DNA computation studies.

DNA nanotechnology in dynamic nanodevices and molecular nanomachines

DNA nanotechnology has opened a new era in smart diagnosis, bioimaging, and treatment, thus designing smart delivery vehicles with DNA nanomachines. DNA nanomachines, which are nanostructures that can move at the nanoscale[59], offer unique advantages like easy synthesis, thermal stability, and functionality[60]. They are promising for applications in biosensors, drug delivery, and molecular computation[61]. Several types of DNA nanomachines have been designed with nanoscale control and biocompatibility, including DNA walkers[62], DNA motors[63], DNA gears[63], DNA nanocages[64], and DNA tweezers[65]. In recent years, there has been a notable increase in research and development efforts focused on dynamic DNA origami devices, which exhibit the capacity to switch among multiple stable or semi-stable states. These structures vary in the way they are triggered, the number of states they can access, their speed, and whether the transitions can be reversed[2]. In addition to incorporating shape complementarity as a design principle, that allows switchable nanodevices to exploit a broader range of molecular recognition mechanisms beyond base pairing, enabling more versatile and adaptable functionality[14], a variety of mechanisms based on properties of DNA and in general, oligonucleotides, have been developed to engineer components of DNA nanomachines:

1. Molecular binding such as the toehold-mediated strand displacement (TMSD) method in DNA tweezers (Fig.3A)[66], [67], [68], and aptamer binding in a logic-gated nanorobot (Fig.3B)[69]. In another study, conformational changes were investigated via releasing or locking strands in a reconfigurable DNA origami tripod with gold nanorods[70]. It can be also exploited in changing the morphology in the nanoscale by triggering strands (Fig.3C)[71]. Catalytic actuation by the activity of an enzyme attached to different arms of a DNA tweezer can also help in designing nanoreactors[72]
2. Environmental stimuli such as reversibly salt-based actuation, capable of response times in the range of hundreds of milliseconds (Fig.3D)[73] or a DNA origami nanoactuator that is responsive to the ions, proteins, or nucleic acids stimuli (Fig.3E)[74], pH-based actuation in a reconfigurable DNA Origami nanocapsule[75], optical-based actuation in a DNA origami sphere via incorporation of fluorescence-cleavable spacers (Fig.3F)[76] or hybridization and dehybridization of azobenzene-modified DNA oligonucleotides with chiral properties via UV/VIS stimuli[77]. The

chiroptical properties have been also investigated in biosensing engineering by DNA nanotechnology[78]

3. External field actuation such as reversibly thermal actuation in a DNA nanovalve that exhibits a bulk response time in the range of tens of minutes (Fig.3G)[79], electric field actuation in a switchable controlled rotating robotic arm on order hundreds of milliseconds (Fig.3H)[80], magnetic actuation in a DNA origami assembly of stiff micro-levers with adjustable rotational speed and a rapid response time in the range of hundreds of milliseconds[81]. In addition to conformational changes, the conductivity of the DNA origami nanopores can be regulated by the applied voltage (Fig.3I)[82], [83], [84]

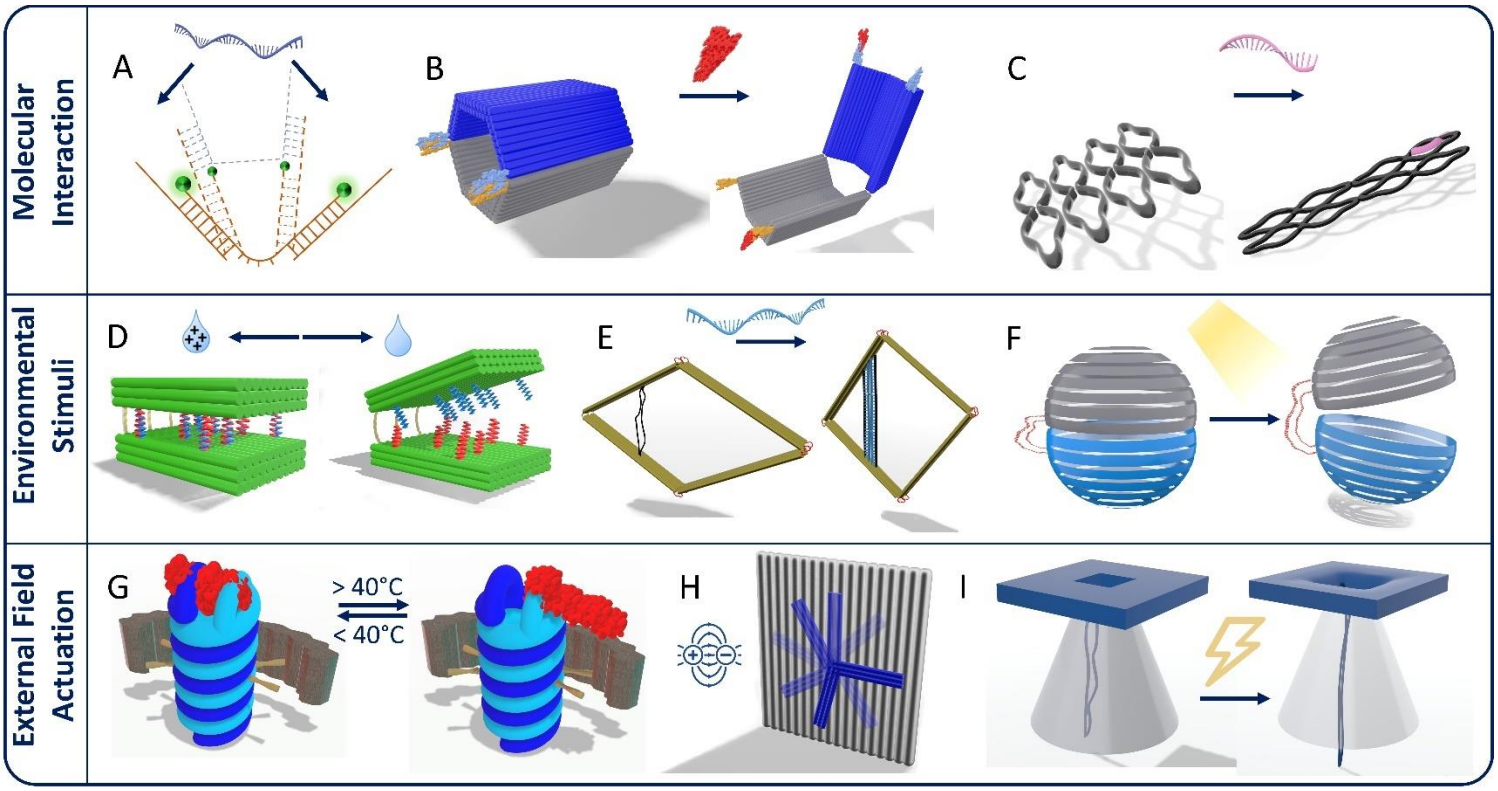


Figure 3- mechanisms applied in dynamic DNA nanodevices: 1. Molecular interaction with a trigger e.g. A) a DNA tweezer that can be changed from close to open by the addition of an oligonucleotide strand[67], B) An automomous DNA nanorobot, regulated by a logic gate encoded in an aptamer, can be maintained in a separated state through its interaction with an antigen key[69] and C) a rectangular array of double-stranded DNA that switches into the alternative stable configuration through a "domino array" transformation when it is triggered by an oligonucleotide strand. 2. Environmental stimuli e.g. D) a DNA origami hinge with single-stranded DNA overhangs sensitive to cation concentrations in solution which results in the hybridization of DNA overhangs closing and opening transitions occur on the millisecond time scale[73], E) A rhombus-shaped DNA origami "nanoactuator" whose shape could be accurately adjusted using "strut-locking" strands, transitioning from a closed configuration to an open configuration by modifying the length of the connecting struts[74], and F) a DNA origami sphere that can be transformed into two tethered hemispheres using light to trigger photo-cleavable spacers within the short oligonucleotide strands connecting adjacent helices[76]. 3. External field actuation e.g. G) a multifunctional nanovalve that is closed at ambient temperatures and its programmable thermosensitive lid opens the barrel above $40^{\circ C}$ to allow transport of small molecules across the membrane[79], H) a DNA-based square with an integrated robotic arm that can be switched by externally applied electrical fields between arbitrary positions within milliseconds, and I) a DNA origami nanopores with a long double-stranded DNA leash combined with glass nanocapillaries that can have conformation change under applied voltages[82]. Figures were sketched with Vectary.

All of these recent advances in DNA nanotechnology have shown great promise in creating encoded machines with complex molecular structures and machine-like functions[85] Due to

the high programmability and versatility of the DNA that can be engineered to self-assemble into complex structures and act as a scaffold for other molecules to bind to[1], [86], the custom DNA nanostructures have immense potential for applications across various fields, including biology, chemistry, and engineering by a wide range of DNA-based nanodevices, including motors, switches, sensors, and more[85]. Thus, they could be used as tools for both studying the behavior of living systems[87] and developing new drug delivery systems that can be actuated and move and target specific cells or tissues. A prominent prototype of such a drug carrier is a logic-gated origami hexagonal barrel, containing antibody fragments, locked with aptamers to hold the two halves together. When the aptamer target, such as a cell receptor, binds to the aptamers, it acts as a lock and key, and the barrel unlocks and exposes its antibody cargo. This enables the antibodies to bind to a different set of receptors, as demonstrated through cell labeling and receptor-mediated signaling pathways[69].

DNA nanotechnology holds the promise of catalyzing a revolutionary shift in the realm of molecular machinery and motors. In addition to switchable nanodevices between two equilibrium states upon actuation, it is feasible to design advanced nanomachines that use external energy, such as a catalytic induce, to move away from equilibrium and shift continuously between various mechanical states. By regulating the amount of energy absorption and encouraging the desired transition, work, and change in the environment become possible[2], [88]. To achieve this goal, DNA-based materials must be integrated with other simple nanodevices to create well-run nanomachines[83], such as biological motor enzymes in bio-hybrid DNA rotor–stator nanoengines[89] or motor proteins in novel nanomotors constructed through DNA origami. These nanomotors are capable of continuously converting chemical fuel into mechanical motion, linearly, and can be tuned, with speeds measured in nanometers per second, and they can travel distances of several microns[90].

Recent impressive developments in DNA nanotechnology have led to significant progress in sensing, imaging, and mechanical devices[85], [91], [92]. As a result, there is an optimistic outlook that the creation of self-sufficient vehicles, capable of identifying issues within the body and taking appropriate action, may soon become feasible[2]. Two DNA nanomachines detected pH changes in specific parts of cells using two different pathways, providing real-time monitoring of early endosomes and the trans-Golgi network. They worked independently and accurately captured the pH of their target organelles[93]. The recent breakthroughs in DNA nanotechnology have enabled the realization of DNA nanorobots that can execute pre-programmed functions. By integrating sensory, controller, and actuation modules, these DNA nanorobots demonstrate the ability to execute diverse mechanical motions in response to specific stimuli. DNA nanorobots possess a wide range of functionalities, such as targeted drug delivery, precise biosensing, and the capacity to autonomously influence cellular behavior according to specific requirements[94]. Researchers created an autonomous DNA nanorobot using DNA origami that can deliver payloads to tumors. Incorporating thrombin and a nucleolin-targeting aptamer, the nanorobot utilized a single entity that acted as both a targeting domain and a molecular trigger. It induced intravascular thrombosis and inhibited tumor growth in tumor-bearing mouse models. In both mice and miniature pigs, the nanorobot demonstrated a safe profile and did not elicit any significant immunological responses[95]. DNA origami is being investigated as a platform to enable the development of in vivo interactive nanorobots and trigger the activation or deactivation of molecular payloads through logical outputs. After successful ex vivo experimentation, researchers advanced to using the DNA origami robots within live cockroaches to exert control over a molecule that selectively targets their cells[96].

Recently, a very remarkable DNA nanorobot has been introduced that not only can respond to stimuli but also can do the first act autonomously and remotely to study the mechanical activation of membrane proteins. The challenges in understanding mechanotransduction, which is the conversion of mechanical forces into biological signals in cells have led to presenting the 'Nano-winch', an innovative molecular actuator based on DNA origami, capable of simultaneously manipulating multiple mechanosensitive receptors in parallel. The Nano-winch can exert low-piconewton forces on cell membranes in autonomous and remotely activated modes, which allows for the study of cellular mechanical processes. The article also demonstrates the application of the Nano-winch in observing the opening of a force-responsive gated channel protein in a single-channel bilayer experiment. The Nano-winch is a customizable tool that can be used to explore various mechanotransduction circuits on living cells without requiring instrumentation[97].

These studies demonstrate that DNA nanotechnology allows for the creation of logical nanorobots that can selectively target and treat tumors[98] with minimal external intervention compared to conventional delivery methods.

DNA computation

DNA computation, alternatively referred to as biomolecular computing, investigates the utilization of DNA molecules as a foundation for computational processes. DNA molecules, with their ability to store and process information, have been proposed as a potential alternative to traditional electronic computers. The fundamental concept driving DNA computation involves harnessing the inherent properties of DNA molecules to perform particular computations. DNA molecules are made up of four basic building blocks, called nucleotides, which can be arranged in different sequences to encode information[99], [100]. Following Adleman's proposal of DNA computation in 1994, which depended on the thermodynamic equilibrium of base pairing and successfully addressed the Hamiltonian path problem [99], and later, the significant advancements in merging digital logic with DNA molecules to construct DNA logic gates, as demonstrated by Okamoto et al. [101], Caltech and IBM scientists, in 2009 [102], used self-assembled DNA scaffolding, based on DNA origami, to build tiny circuit boards, on surfaces that were compatible with today's semiconductor manufacturing equipment.

DNA's double helix structure and base pairing allow for the encoding of information, which can be converted to various forms under external conditions. The potential of DNA computing for use in biomedical and biological diagnostics has been investigated. While DNA-based logic systems can perform computations, similarly to analog computers, such as cascading, signal storage, feedback, and amplification, they are not yet as powerful as current semiconductor computer technology due to limitations in nucleic acid molecules. However, The application of DNA computation in diagnostics brings forth several benefits, including the ability to integrate biomarker recognition with the analysis and reporting of results, programmable logic for more accurate results, and the ability to interface with traditional biosensing and clinical diagnostics[100]. There are several ways to construct a DNA-based computing device, but most of them rely on DNA as the foundation to create the fundamental logic gates of digital logic, such as AND, OR, and NOT gates. Construction of DNA logic gates could be based on DNAzymes[103], [104], strand displacement mechanisms[105], toehold exchange[106], chemical reaction networks (CRNs)[107], enzymes[108], or algorithmic self-assembly[109], [110].

Scientists have developed knowledge about the structure and functions of cells to create molecular computers that use nucleic acids. They have explored methods to engineer logic

gates using DNA and RNA and other forms of oligonucleotides, both in laboratory settings and within living cells. Some examples of these methods include using DNA origami to create nanorobots for performing logic operations in vivo[69], transcriptor technology to control transcription rates in vivo[111], and various other techniques involving DNA aptamers[69], tandem riboswitch core machinery[112] or ribozymes[113] to engineer logic gates in vivo[114], [115].

Although there are some limitations regarding DNA computing, it has made many advantages over traditional computing including improved performance due to the ability to run millions of operations simultaneously, massively parallel processing that can enhance computing speed by 10,000 times, high storage capability with 1 cubic nanometer required for one bit, and minimal power consumption due to the lack of requirement for electricity in chemical bonds. DNA computing is particularly useful for problems involving a large number of calculations, data storage, cryptography, and steganography[116].

Recently, A new system has been developed that combines DNA computing with CRISPR technology and displays it digitally. This system utilizes CRISPR's ability to recognize and cut specific DNA sequences, employing predetermined DNA targets as input and a fluorescence signal that switches ON or OFF as the output. This arrangement enables a direct mapping between input and output, facilitating the implementation of multilevel DNA logic computing. Researchers have advanced the potential of CRISPR technology by employing pre-CRISPR reactions, expanding input size, and enhancing computing capabilities. With digitally displaying by microfluidic techniques, this breakthrough offers applications in DNA molecular encryption, cryptography, and steganography allowing for the encryption and decoding of digital inputs and message outputs through programmed molecular patterns on a substrate. The flexibility of the CRISPR system enables diverse input possibilities for real-world information encoding. Overall, this CRISPR-powered DNA computing system offers a simplified approach to large-scale molecular programming and has the potential to revolutionize DNA computing[117]. It seems that development in CRISPR-powered DNA computation may show promise for applications in gene programming, editing, and modification of genetic disorders.

A significant advancement in electronic integrated circuits history was the invention of field-programmable gate arrays (FPGAs), semiconductor devices enabling post-manufacturing reprogramming of integrated circuits. FPGAs consist of logic blocks arranged in a grid and linked by customizable routing paths, akin to programming software, facilitating the creation of custom circuits. Inspired by silicon-based FPGAs, researchers have recently developed DNA-based programmable gate arrays (DPGAs) with high scalability. They showcased a practical application of DNA integrated circuits (DICs) by reconfiguring and programming multilayer DPGAs to design a quadratic equation-solving DIC. This liquid computer could run more than 100 billion distinct circuits. Additionally, to demonstrate the clinical significance of DICs, the researchers tackled a crucial issue related to molecular circuits for identifying disease-associated biomarkers. They developed a nonlinear classifier essential for tasks such as decision trees and clustering. Their system successfully distinguished between healthy and diseased samples, accurately identifying all 23 samples tested within about two hours[118]. This grants DNA drug carriers diagnostic capabilities far surpassing those of conventional designs, solely reliant on stimuli-responsiveness. Through this approach, DNA carriers can accurately pinpoint tumor sites by discerning and differentiating them from healthy tissues through computational means.

Another related field harnessing the encoded properties of DNA is data storage. The shortcomings of conventional storage techniques and the promise of DNA-based data storage as a viable alternative have resulted in a vast scientific investigation into bio-data storage. This

approach involves employing DNA synthesis, sequencing, and encoding algorithms to store substantial volumes of data with exceptional density and longevity. This field has gained significant attention in the past decade, with several breakthroughs. DNA storage offers advantages such as stability, ease of replication and backup, limitless uses, and the ability to survive harsh conditions. Theoretically, each gram of DNA can store a vast amount of digital information, and it can withstand extreme temperatures when appropriately protected. These unique features make DNA a promising material for secure and long-term data storage in the future[119]. In 2019, a DNA data storage system was created which employs software developed by the joint efforts of Microsoft and the University of Washington team. This innovative system was able to transform the binary code of digital information into the nucleotide bases (A, T, C, and G) that constitute the fundamental elements of DNA[120]. A recent demonstration showcased a technique for conducting searches for similarities within a DNA-based database comprising 1.6 million images, using binding probes and a conversion of images to genetic sequences to execute queries. Experimental findings indicate that this molecular technique demonstrates similar performance to cutting-edge computational algorithms for conducting resemblance searches[121].

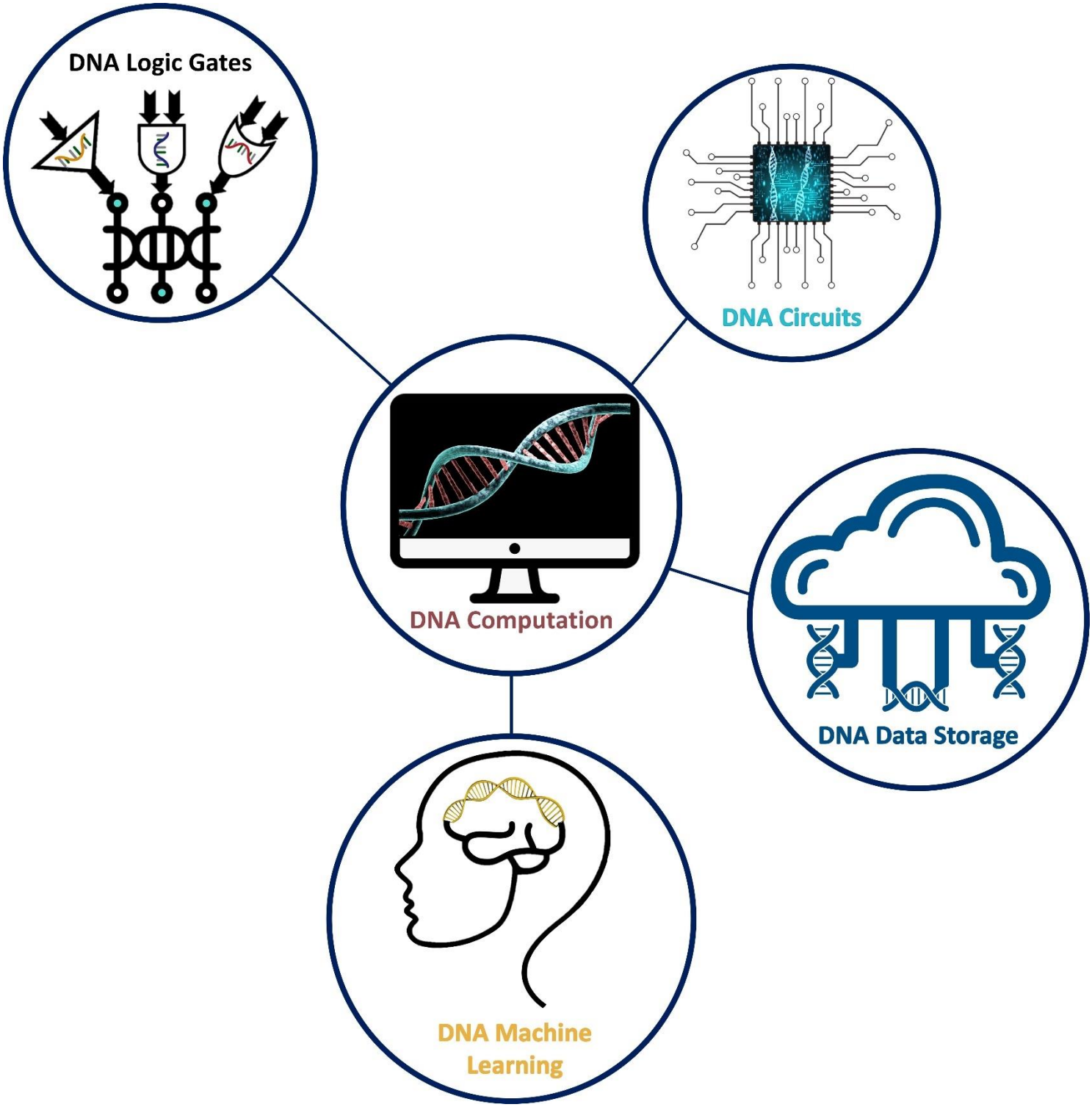


Figure 4- The field of DNA computation began with basic DNA logic gates and has progressed towards creating increasingly complex circuits, culminating in the development of commercial DNA data storage solutions. Although DNA machine learning is still in its infancy, there is potential for its advancement toward the creation of future 'bio-AIs'.

Meanwhile, DNA nanotechnology in the field of data storage shows promise in addressing challenges associated with synthesis and reading. By leveraging the self-assembly properties of DNA strands, this technology enables the construction of intricate 2D and 3D architectures. Various methodologies, including DNA tile assembly or DNA origami, facilitate the fabrication of nanoscale forms and designs. These structures can store data in their three-

dimensional shape rather than the sequence of bases. The non-covalent nature of DNA allows for easy reconfiguration using techniques like strand displacement, thermal annealing, and pH changes. This reconfigurability enables data erasure and rewriting without the need for chemical synthesis. Additionally, DNA nanotechnology offers the potential for encryption and data operations similar to computer memory systems. While data storages based on DNA nanotechnology fall short in terms of data density when in comparison with nucleotide sequence itself in encoding data, it still outperforms existing hard drive technologies by a significant margin. Moreover, there is room for improvement by fine-tuning three-dimensional DNA structures to achieve even higher data densities. Overall, DNA nanotechnology shows promise for data storage with advantages in readout, simplicity, and reconfigurability[122]. It has been suggested that by synergistically integrating cutting-edge molecular biological tools such as CRISPR and DNA nanoengineering, remarkable advancements can be made in the domains of bio-computing and bio-storage. This integration offers improved speed and efficiency in the procedures of encoding and decoding DNA information. Additionally, advancements in synthesis and sequencing capabilities contribute to increased throughput and cost reduction, making large-scale DNA-based data storage platforms more practical. It is estimated that accelerated progress in DNA-associated technologies could be a great answer to the problem of data explosion[119].

The DNA data storage market is anticipated to witness substantial growth, commencing from a value of USD 76 million in 2024 and projected to escalate to USD 3,348 million by 2030, with an impressive compound annual growth rate (CAGR) of 87.7%. This upward trajectory is propelled by the burgeoning demand for data storage. Leading participants in the DNA data storage sector include Illumina, Inc. (US), Microsoft (US), Iridia, Inc. (US), Twist Bioscience (US), and Catalog (US)[123]. In 2022, Catalog (US) and Seagate Technology LLC (Ireland) announced a collaboration aimed at advancing DNA-based storage and computation platforms. Their joint efforts seek to reduce platform sizes significantly, potentially up to 1000 times, leveraging Seagate's "lab on a chip" technology. This collaborative research explores the potential for future DNA-based storage and computation platforms across various form factors, ranging from desktops to Internet of Things (IoT) applications[124]. Iridia, Inc., a nanotechnology firm, has pioneered a groundbreaking technology that merges DNA polymer synthesis, semiconductor fabrication, and electronic nano-switches to facilitate high-density data storage, rendering it commercially viable. Through a fusion of proprietary DNA synthesis chemistry, unique hardware architecture, and semiconductor fabrication techniques, Iridia develops state-of-the-art solutions for seamlessly writing, storing, and retrieving data at extraordinary densities[123].

The area of computer science that focuses on constructing and operating DNA-based computers represents a field of study that investigates the utilization of DNA as both hardware and software for advanced, tailored, and precise diagnostics in biomedicine. One of the key elements of this approach is using machine learning techniques to retrieve specific DNA sequences, which are then used to design smart diagnostic biochip projects. In one of the recently introduced technologies, the biomolecular queue automata strategy allows for the arrangement of molecular dimensions of computational operations by employing sequential actions of cleavage and ligation of DNA molecules. The incorporation of enzymes is of significant importance in the design of biomolecular computers with memory, enabling their functionality. The authors also introduced the Queue-PCR method, which employs biomolecular computers, and automates the PCR process. During the design phase of these biochips, machine learning techniques play a vital role, highlighting the potential application of enzymes in constructing diverse data formats akin to those in computer science[125].

DNA computation currently faces challenges in competing with silicon chips due to its much lower computation speed. However, its potential in biomedical applications, particularly in the development of bio-nanorobots, is promising. These bio-nanorobots could excel in tasks such as assessment and operational calculation within biological systems, offering advantages over conventional delivery carriers. Their ability to perform these tasks could indeed be more advanced, aligning with the concept of "smart" technology. While realizing this potential may still be in the future, ongoing advancements in DNA computing and nanotechnology suggest that such applications could eventually become feasible.

Combination of DNA computation with dynamic nanodevices: prospects in bio-nanorobots in precision medicine

Recent groundbreaking advancements in rational DNA-programmed nanomaterials for biomedical applications, such as biosensing, bioimaging, drug delivery, and therapy[32], have the potential to initiate a significant transformation. The design and production of DNA nanocomputing circuits have been evolving in recent years, even utilizing microfluidic platforms[126]. It is now possible to estimate the feasibility of producing future DNA computers, whether at nano or other small scales, in vitro, inside cells, or even in vivo. This opens up exciting possibilities for the development of 'bio-nanorobots' in precision medicine.

What sets this type of bionanorobot apart is its programmability and intelligent controllability, along with its high versatility due to functional biological components. A DNA platform nanorobot, integrated with other modules such as CRISPR, may be able to explore cellular processes, detect mutations caused by cancers or genetic disorders, interact with molecules, make feedback to external controllers, and deliver medicine within the body. While there have been recent advancements in developing biomedical nanorobots using alternative platforms[127], [128], the distinctive features lie in their ability to be programmed and controlled externally, as well as their versatility in utilizing functional biological components. Some of the recent prototypes of this model would be a DNA nanorobot that possesses intelligence and is designed to autonomously prevent blood clotting in human plasma. The nanorobot consists of a cylindrical DNA structure as the main framework, with embedded molecular reaction cascades serving as the computational core. By intelligently detecting the level of thrombin in the immediate surroundings, the nanorobot can initiate anticoagulation on its own when there is an excessive amount of thrombin present. The specific concentration of thrombin that triggers the nanorobot's response can be adjusted as desired to prevent potential side effects caused by an excess of thrombin. This capability allows the nanorobot to be applicable in various medical scenarios for autonomous anticoagulation, providing inspiration for a more effective and safer approach to personalized medicine in the future[129]. While in the delivery of oligonucleotide drugs, the nanorobot should detect the targeted cells and go towards them by movement tools, it may be possible to introduce certain functions or algorithms that, when calculated by the nanorobot, will allow it to attach to or enter the cell (Fig.5A) and after affecting the cell and detecting the response, the nanorobot may calculate the reverse function or other algorithms to detach from or exit the cell (Fig. 5B) to prevent the excess accumulation of the nanorobots in the cells. This bio-nanorobot, equipped with a DNA-based Central Processing Unit (CPU) as its control center and a sophisticated dynamic device complex as its physical structure, serves dual purposes as both a diagnostic and therapeutic tool. It stores individual genome data in its memory to detect gene mutations. Utilizing its computational capabilities, it can distinguish diseased tissue from healthy tissue, offering superior accuracy and safety compared to conventional drug carriers that react passively to system stimuli. Unlike passive carriers, the DNA nanorobot actively engages with molecules or cells, executing operations and effectively delivering the oligo drugs (Fig. 5C). Integration

with biological components like CRISPR/Cas9 enables the editing of problematic genes after entering the nucleus (Fig.5D). Through precise DNA cutting and leveraging natural repair processes, it can deactivate or treat cancerous cells rather than simply eliminate them[130], thereby bolstering the body's immune response against cancer.

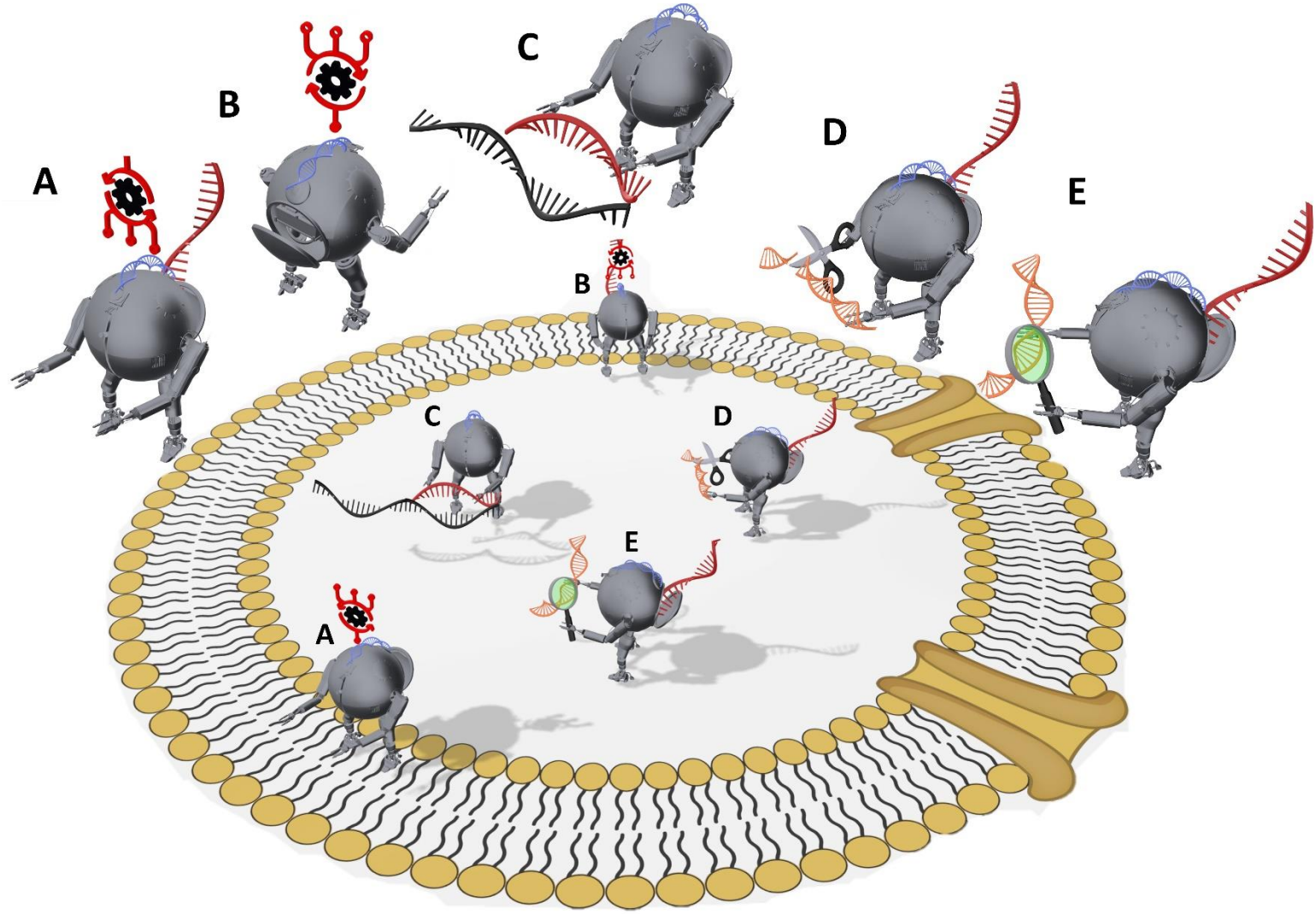


Figure 5- The utilization of DNA nanorobots in the treatment of cancerous cells. These nanorobots have the capability to penetrate into the cell (A) and exit it (B), including potential entry into the nucleus, possibly through a programmed algorithm for entry and its reverse for exit. They can effectively transport oligonucleotide drugs to specific targets within the cell (C) and may also possess gene-editing capabilities like CRISPR/Cas9 (D), enabling them to modify genetic material. Moreover, it is envisaged that with the integration of artificial intelligence, these bio-nanorobots could analyze individual genome data, identify potential errors (E), and subsequently amend or even rewrite the targeting oligonucleotide drug to enhance efficacy.

Ongoing exploration of the machine-learning capabilities of DNA computers[131], [132], along with the utilization of additional components such as enzymatic neural networks[133], holds promise for empowering decision-making abilities within the biorobot. This means that if the biorobot carries nucleic acid drugs, it can adaptively reprogram them using its programmable gate arrays in response to further mutations resulting from genomic instability, adjusting the drugs as needed for treatment. These advancements might also facilitate alterations to the DNA vehicle itself while inside the body, allowing this compact DNA-based "bio-AI" to autonomously reprogram in response to changes in the tumor microenvironment or

other bodily incidents. It could continuously monitor the body's health throughout its residency (Fig. 5E), even years post-treatment, to prevent cancer recurrence.

Conclusion

The incidence of all types of cancer is estimated to increase up to 2040, for all ages and sexes[134]. Thus, more efficient measures should be tackled in prevention, diagnosis, and treatment. DNA's programmability and interactions with other molecules make it versatile and easily controlled. It has potential in tissue regeneration, disease prevention, and drug delivery. Meanwhile, the DNA origami method shows promise for designing and delivering drugs, particularly for oligonucleotide therapeutics that enable precision medicine for cancers and even rare genetic diseases. To optimize its utility, the production procedure of the DNA origami platforms should be streamlined by reducing the need for extensive purification of oligonucleotide strands. This could be achieved by reconfiguring the production method to occur intracellularly rather than via chemical synthesis.

While DNA-based nanomaterials have shown acceptable results in diagnosis and drug delivery[32], we suggest that intelligent bionanorobots could also be designed in such a way as to reflect the genetic information to the outside, and thus the possibility of the mutation could be anticipated. Creating such a cutting-edge bio-nano-AI necessitates the collaboration of diverse experts across fields such as molecular biology, biochemistry, genetics, computer science, and engineering, as well as interdisciplinary areas like bioinformatics and biomedical engineering.

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