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Mini-Review

Nucleic Acid Architectonics for pH-Responsive DNA Systems and Devices

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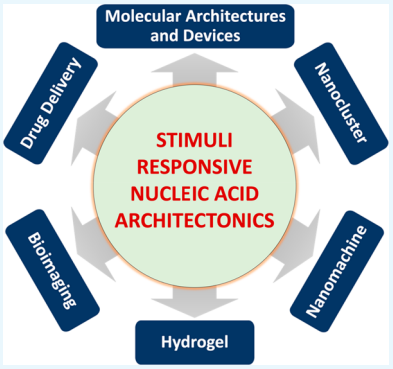
ABSTRACT: Nucleic acid-based architectures have opened up numerous opportunities for basic and applied research in the field of DNA nanotechnology. The scheme of molecular architectonics of nucleic acids exploits conventional and unconventional base pairing interactions to integrate molecular partners in constructing functional molecular architectures and devices. The pH-responsive functional nucleic acid systems and devices have gained interest in diagnostics and therapeutics because of their biocompatibility and structural programmability. In this Mini-Review, we discuss recent advancements in the area of nucleic acid architectonics with a special emphasis on pH-driven molecular systems including molecular and nanoarchitectures, templated architectures and nanoclusters, nanomachines, hydrogels, targeted bioimaging, and drug delivery architectures. Finally, the Mini-Review is concluded by highlighting the challenges and opportunities for future developments.



INTRODUCTION

Nucleic acids adopt diverse molecular architectures that play a key role in storing, processing, and transmission of genetic information. Over the past few decades, researchers have been engaged in programming the molecular organization of DNA for varied applications in material science.[1] The nucleobases, namely, adenine (A), guanine (G), cytosine (C), and thymine (T), form A:T and G:C base pairing through Watson−Crick (WC) hydrogen bonding to form a DNA double helical structure. The N-H groups of the nucleobases are potent hydrogen bond donors and sp^2-hybridized electron pairs on the oxygen of carbonyl group and the ring nitrogen are hydrogen bond acceptors, which are involved in canonical (WC) and noncanonical (non-WC) hydrogen bonding in the form of A:T, G:C, A:A, and A:G interactions (Figure 1A−C).[1,2] The ability of A to form A:T, A:A, and A:G pairs has inspired the development of hybrid DNA ensembles.[3−5] Apart from WC and non-WC hydrogen bonding, guanine-uracil (G:U) base pairing (wobble base pair) play a crucial role in the RNA architectures. The structural studies of yeast tRNA showed that the G:U wobble pair has comparable thermodynamic stability to that of WC base pairs, and therefore, it is frequently used to replace G:C or A:U base pairs, resulting in RNA secondary structures such as helix, stem-loops, and pseudoknots (Figure 1D).[1] DNA is preferable over RNA for nanomaterials applications because of its higher stability and low cost of synthesis, while RNA is known for structural diversity. Further, the high-fidelity hydrogen bonding and structural programmability makes DNA a superior molecular system compared to small molecules, peptides, and polymers (Table 1). The replacement of classic WC base pairing interaction with metal complexation is another important development and metal base pairing interaction potentially generates DNA-templated nanoarchitectures.[1] It has been shown that a silver ion (Ag^+) exhibits strong affinity toward C and selectively forms C-Ag^+-C complexation, while a mercury ion (Hg^{2+}) binds to T to form a T-Hg^{2+}-T complex (Figure 1G).[1] Under physiological conditions, the G-rich region of genes (e.g., telomeres) forms a four-stranded noncanonical DNA structure known as the G-quadruplex (GQ). GQ structures formed through Hoogsteen hydrogen bonding in the form of a tetrad of four Gs, which is further stabilized by metal ions such as K^+ and Na^+.[2] These diverse nucleic acids structures can serve as powerful tools and methods in the custom design of molecular and material architectures with functional properties and applications through the scheme of molecular architectonics.[6,7]

The new era of DNA nanotechnology allows exploitation of stimuli-triggered conformational change, reorganization, or reconstruction of DNA nanoarchitecture as signal readout mechanisms. The protonation of nucleobases elicits non-WC base pairing interactions such as Hoogsteen hydrogen bonding, which are strongly dependent on pH conditions. The

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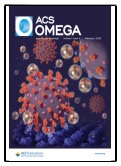

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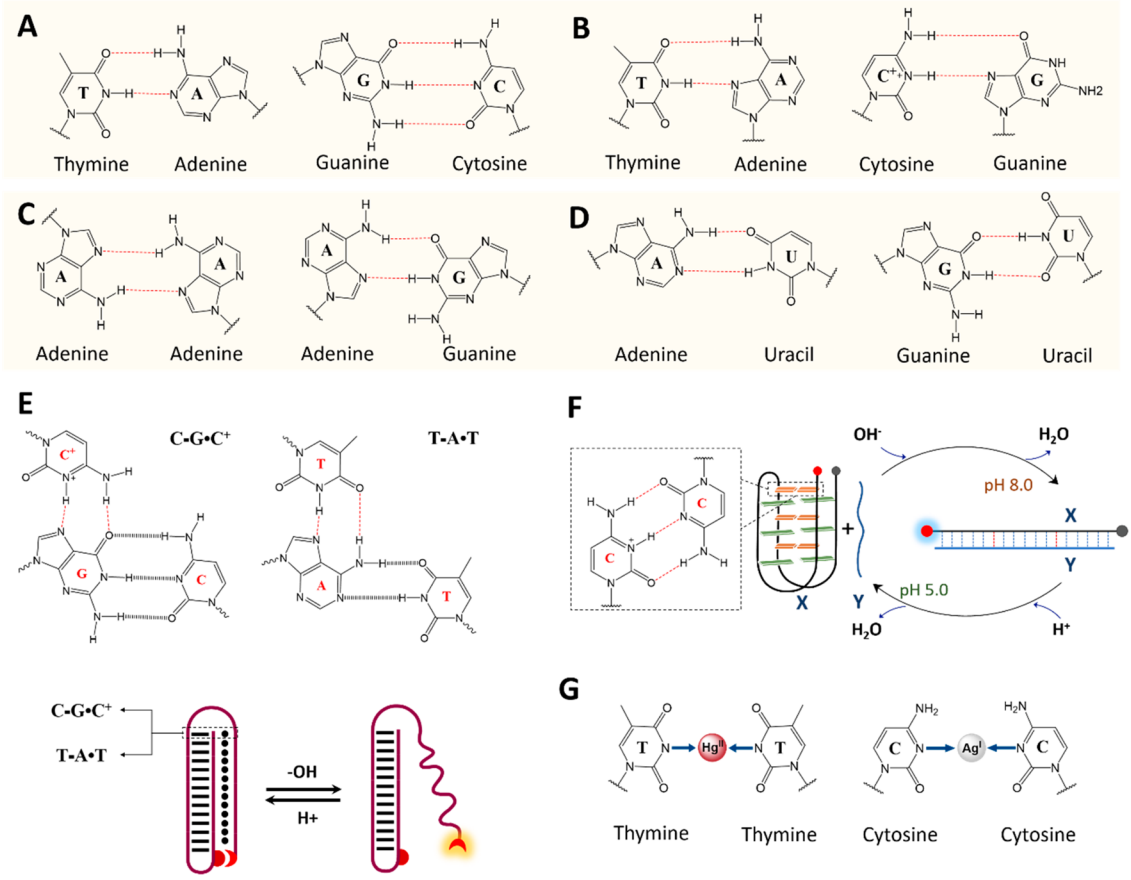

Figure 1. (A) Watson and Crick (WC) hydrogen bonding scheme for adenine-thymine (A:T) and guanine-cytosine (G:C) base pairs. (B) Hoogsteen hydrogen bonding scheme for A:T and G:C base pairs, (C) noncanonical hydrogen bonding for A:A and A:G, (D) Wobble base pair of A:U and G:U (E) pH-dependent DNA triplex formation via WC (dashed) and Hoogsteen (dots) interaction.[9] (F) A reversible duplex to *i*-motif transition in the DNA switch.[10] (G) Noncanonical DNA metal complexation (T-Hg^{II}-T and C-Ag^{I}-C).

Table 1. Comparison of Nanoarchitectures Designed from Nucleic Acids and Other Molecules

		pros	cons
nucleic acid	DNA	high stability, programmable, biocompatible, non-toxic, ease of synthesis and modification, and well-established pH-dependent secondary conformations	limited stability toward extreme pH and temperature
	RNA	diverse secondary and tertiary conformations	highly unstable, expensive, and sensitive to RNases
other nanomaterials	small molecules	bioavailability, metabolic stability, small size, ease of synthesis and broad tunability	unique properties and pH dependency of small molecules cannot be generalized
	peptides/proteins	high potency and selectivity, high chemical and biological diversity, ease of synthesis and structural tunability, and pH-responsive nature is reasonably established	poor metabolic stability, weak membrane permeability, low oral bioavailability and predictable behavior toward pH
	polymer	alternative for nucleic acids, small molecule and protein-based nanomaterials	limited tunability, complex procedure for synthesis and processing, high cost, and pH response not well-defined

formation of characteristic DNA secondary structures such as three-stranded triplex through binding of a third strand in the major groove of duplex and four-stranded intercalated motif (*i*-motif) under acidic pH conditions are being utilized in designing pH-responsive DNA molecular and nanoarchitectures. A DNA triplex nanoswitch was designed with a self-complementary sequence and excellent programmability (Figure 1E). Initially, a pH-responsive duplex DNA consisting of a single hairpin was formed by WC (dashes) hybridization of two sequences of 10 complementary base pairs separated with a five-base-pair loop. Interestingly, a second hairpin is formed due to a change in pH through Hoogsteen parallel interactions (dots) with the other end of the switch, which enables the duplex DNA to form a triplex structure. This study was carried out by two different types of triplex content mainly T-A·T and C-G·C^+ sequences, wherein the N^3 of C in the third strand undergoes protonation to form C-G·C^+ parallel triplet around pH 6.5. The T-A·T triplet is significantly stable at neutral pH, while it is unstable at pH 10 because of deprotonation. To understand the duplex to triplex transformation, the system was labeled with a fluorophore (donor: Alexa Fluor 680) and a quencher (acceptor: BHQ-2). The fluorophore is conjugated to one end of the DNA sequence, and the quencher is introduced between the loops of hairpin DNA. As the structure unfolds (triplex-to-duplex transformation), fluorophore moves away from the quencher resulting in an enhanced fluorescence signal. The fluorescence signal reveals the formation of a duplex structure, while

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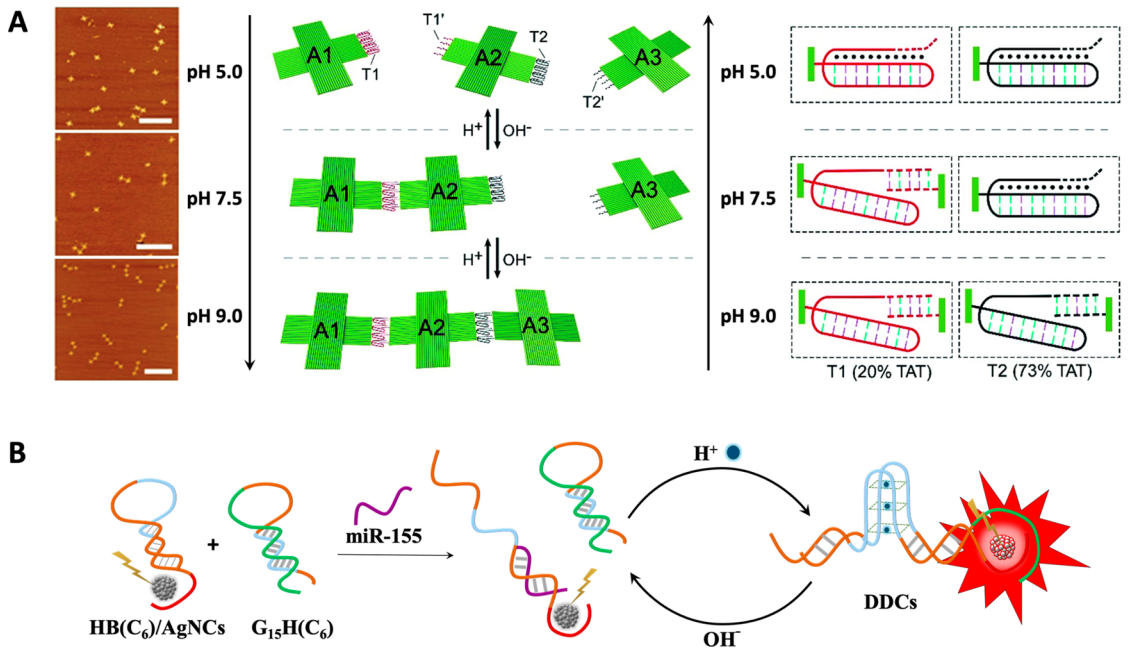

Figure 2. (A) pH-responsive assembly/disassembly of DNA architectonics (respective AFM images of monomer−dimer−trimer). (B) pH-regulated cyclic process of duplex DNA constructs and fluorescence of HB/AgNCs in the presence of miR-155. 2A reproduced with permission from ref 11. Copyright 2019 The Royal Society of Chemistry. 2B reproduced with permission from ref 14. Copyright 2020 American Chemical Society.

fluorescence quenching corresponds to the formation of triplex nanoswitch.[9] Similarly, a unique duplex molecular system was constructed that can switch its conformation to an *i*-motif upon pH variation, which consists of an ssDNA sequence X component with four stretches of three Cs, and a single-stranded partially complementary DNA sequence Y (Figure 1F). The DNA system protonates at pH 5.0 and forms C·CH$^+$ base pair with X folding into a four-stranded *i*-motif conformation. When the pH is raised to 8.0, the X unfolds and becomes an extended duplex structure XY. The X sequence is attached with a donor and acceptor fluorophore to understand the switching mechanism of nanomachine by interchanging the pH conditions.[10] Apparently, templated assembly of functional molecules or any designed structural units and short DNA oligonucleotides (dB_n, where B = nucleobase, n = length of DNA) offers simple and cost-effective means of constructing DNA nanosystems and architectures.[1] Modulation of canonical and noncanonical hydrogen bonding in response to pH stimulus results in predictable conformational changes, which can be utilized as the underlying design principle for the construction of functional nanoarchitectures and devices. These design strategies offer exceptional programmability, customizability, and addressability to DNA nanoarchitectures. This Mini-Review discusses stimuli-responsive nucleic acid systems with a wide range of applications. Specifically, we cover recent works on pH-responsive DNA molecular and nanoarchitectures, DNA-templated nanoclusters, DNA nanomachines, DNA-based hydrogels, DNA targeted bioimaging, and DNA architectonics-guided drug delivery.

pH-DRIVEN DNA MOLECULAR AND NANOARCHITECTONICS

The scheme of molecular and nanoarchitectonics is a game-changer in taking forward the field of DNA nanotechnology with a huge impact on the advancement of science and technology.[1,8] The fine-tuning of structure and associated properties through simple and reproducible molecular interactions of DNA and functional molecules is the essence of nucleic acid architectonics to generate robust and cost-effective molecular and material architectures with practical applications.[1] One of the effective and adaptive methods of creating advanced DNA nanoarchitectures is through controlling the molecular assembly in response to internal or external stimulus. Wang et al. have optimized pH-regulated DNA origami nanoclusters modified by CGC vs. TAT ratio based on the multistep cyclic self-assembly of DNA triplex as a dynamic linker.[11] The interaction between pH-insensitive ssDNA and WC duplex pairing undergoes the conformational change of triplex-to-duplex transition through pH-sensitive parallel Hoogsteen base-pairing to yield the desired DNA hybridization. DNA origami trimers are formed in the assembly/disassembly of two unique types of intramolecular DNA triplexes upon the pH-stimuli response (Figure 2A). The right arms of tile A1 and A2 are modified with DNA triplex strands T1 and T2 consisting of 20% and 73% of T-A·T composition, respectively. Both oligonucleotide triplexes consist of complementary sticky single-stranded DNA ends (T1′ and T2′, respectively), which are connected to the left arms of A2 and A3, respectively. The A1, A2, A3 (DNA origami monomers) were integrated with 1:1:1 molar ratio at pH 5 in Tris-acetate EDTA (TAE) buffer solution, resulting in the intramolecular triplex formation and the cohesion ability of their sticky ends is prevented, thereby A1, A2, A3 stayed unhybridized in solution. Upon increasing pH from 5.0 to 7.5, a dimer (A1/A2) is formed through the dissociation of T1 on tile A1 by releasing the sticky ends, which leads to the binding of complementary strand (T1′) on tile A2. Becuase of the high T-A·T concentration (73%) in T2, it remains folded leaving the tile A3 monomer apart. A further increase of pH to 9.0 the triplex

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T2 unfolds from tile A2 by releasing the sticky ends which leads to the formation of the trimer (A1/A2/A3) through the association of dimer A1/A2 and monomer A3. Later, the assembly system can be reversed by bringing back the system to a neutral pH condition.

Recently, an electrochemical biosensor was developed by employing DNA zipper architecture which consists of pH-responsive configuration switching DNA lock. The DNA zipper sensing module was functionalized with nine DNA locks, each lock consisting of two parts, DNA hairpin and ssDNA, that are attached to the opposite arm of the zipper to form a triplex.[12] For the open conformation, the ssDNA counterparts of the triplexes were substituted with fragmented DNA sequences that cannot take part in triplex formation. The DNA lock sequence was arranged such that the zipper adopts a closed conformation at pH 6.5 and transforms to an open conformation at 8.0 pH. Further, by switching the buffer conditions between the two pH regimes, it was possible to reliably discriminate between the folding and unfolding configuration of the DNA zipper. Kim et al. developed a framework of DNA nanocage for encapsulation of enzymes wherein one arm of the cage opens up in response to low pH due to formation of *i*-motif providing control over enzyme functions.[13] The *i*-motif forming sequence serves as a gate to regulate stimuli responsive reversible access to enzyme activity. The authors have covalently attached an enzyme inside of the DNA tetrahedron (Td) cage to achieve reversible enzyme activity. In acidic pH, the cage opens up, allowing assess to encapsulated enzyme to the surrounding environment. The opening and closing of the DNA Td cage at two different pH values (6.0 and 8.0, respectively) is confirmed by FRET study employing Cy3 and AlexaFluora 488 fluorescent dyes. This method demonstrates an efficient design of stimuli-responsive opening and closing of DNA nanocage that can encapsulate protein and regulate protein functions, which is also capable of serving as a delivery carrier. The controlled pH-responsive switching triggers the conformational change depending on multilevel assemblies/dissembles of DNA, which may serve as a fascinating property for biomedical applications.

STIMULI-RESPONSIVE DNA-TEMPLATED NANOCLUSTER

The aggregation of metal atoms into a nanocluster exhibits intense fluorescence emission, large stock shift, and good biocompatibility. Several C-rich DNA sequences have been used as scaffolds for the fabrication of DNA-templated silver nanoclusters (DNA-AgNCs) exploiting the strong affinity of Ag^+ to C through C-Ag^+-C complexation. Xu et al. reported G-enhanced fluorescence response of AgNCs for the recognition of miR-155.[14] The design is based on a stem-loop hairpin beacon (HB) encoding with a microRNA-155 (miR-155)-recognizable sequence and AgNCs-template, as well as a hairpin helper tethering a partially locked G-rich tail ($G_{15}H$) and two identical C-rich segments in HB. Because of aberrant expression in cancer, miR-155 was selected as a target, and identical C-rich sequences are introduced for one-half of the *i*-motif tetraplex in two hairpin probes. In PBS buffer (pH 7.0), HB (HB/DNA-AgNCs) was typically nonfluorescent. The addition of miR-155 causes repetitive hairpin assembly of HB and $G_{15}H$ because of the strand displacement at pH 5.0, resulting in a large number of duplex DNA constructs (DDCs) (Figure 2B). Remarkably, at pH 5.0, the constant folding of pH-dependent *i*-motif integrated DDCs causes spontaneous conformational change bringing the G_{15} sequence adjacent to AgNCs which resulted in turn on red fluorescence. The increase of pH to 7.0 caused the reduction of fluorescence intensity, which was further restored by the unfolding of *i*-motif at pH 5.0. Precisely, the intrinsic proximity of Gs can evoke exponential fluorescence enhancement of weakly emissive clusters, the phenomenon is possibly attributed to the electron transfer from G to metal clusters or secondary structure formation in the G-rich segment. This strategy enabled inexpensive, rapid, and highly sensitive detection of miR-155 (LOD 67 pM). The pH-responsive sensing strategies in AgNCs can be suitably extended to specific metal ions capable of changing the oxidation state of AgNCs. Chen et al. developed a fluorescence assay employing Fe(III) as the trigger in a pH-responsive fluorogenic silver nanocluster stabilized by a C-rich-templated DNA sequence.[15] In this work, a fluorescent assay was developed using 12 polycytosine oligonucleotides (dC_{12}) based DNA-AgNCs for sensing Fe(III) selectively among other metal ions. The dC_{12}-templated DNA-AgNCs exhibit pH-dependent fluorescence response wherein emission is quenched in acidic pH and regains upon increasing the pH. The detection of Fe(III) was based on fluorescence quenching of DNA-AgNCs caused by oxidation of AgNCs by Fe(III), which cannot be reversed by adjusting the pH. Meticulous optimization of the working pH to ∼4 resulted in improved sensitivity by 50 folds with a LOD of 3 nM. This assay was capable of detecting Fe(III) with appreciable sensitivity in human serum samples. Thus, the pH-responsive DNA-AgNCs based fluorescence sensing platform holds potential in sensing metal ions in clinically relevant samples.

DNA-BASED NANOMACHINE

Construction of DNA nanomachines involves precise control over stimuli-responsive conformational dynamics to trigger predictable switch between the distinct conformations. The design of the DNA nanomachine requires the understanding of molecular interactions among the nucleobases. Ricci et al. developed a pH-responsive DNA-based triplex nanoswitch, wherein rational tuning of the TAT/CGC content confers a programmable folding/unfolding character to the DNA triplex.[9] Later, a self-propelled biocatalytic micromotor conjugated with DNA nanomachine was developed to detect variation in the environmental pH conditions in real-time.[16] This DNA nanoswitch was modified with Cy5 (acceptor) and Cy3 (donor) for a fluorescence resonance energy transfer (FRET)-based assay to detect specific substrates particularly in the presence of urea. A movable urease-functionalized hollow silica microcapsule is immobilized to a pH-responsive DNA nanoswitch. This allows the real-time examination of pH change in the system that arises from the enzyme reaction responsible for the micromotor's self-propulsion. High-resolution optical tweezers were used to measure the propulsion force of urease-powered micromotors by trapping individual micromotors and measuring their displacement from the trap center, which confirmed that micromotor self-propulsion was reduced over time. However, these self-propelled biocatalytic micromotors were combined with synthetic DNA nanoswitches to detect pH changes in the surrounding microenvironment, and tracking their activity profile was successfully demonstrated.

The pH gradient exists across organelles and organellar pathways within the intracellular milieu and can act as stimuli

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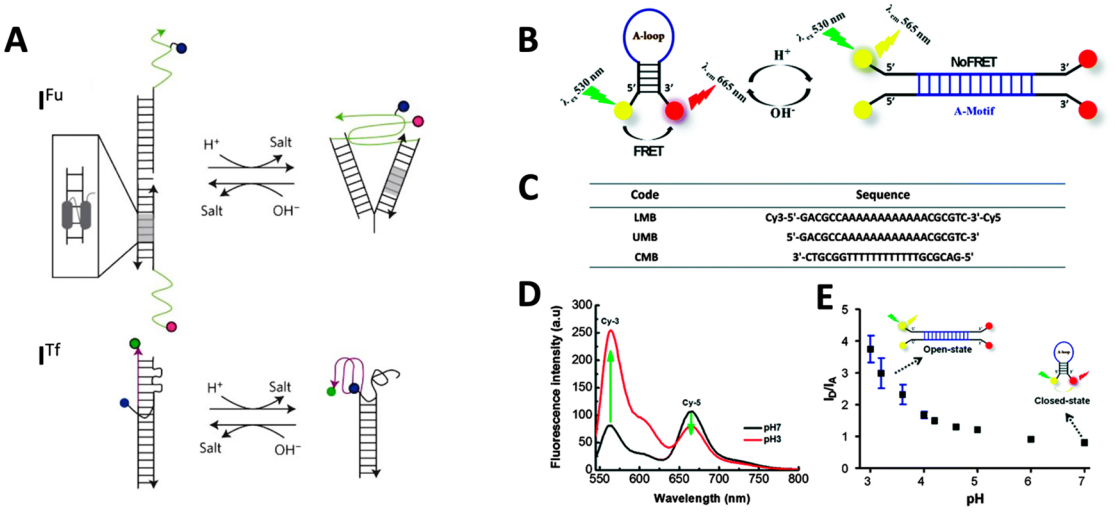

Figure 3. (A) DNA machines that map the furin I^{Fu} (top) and transferrin I^{Tf} (bottom) pathways. (B) pH-triggered A-motif sensor (Ex: 530 nm) (C) DNA sequence used in the study. (D) Fluorescence emission spectra for LMB at pH 3 and 7 under ambient condition. (E) Fluorescence intensity ratio of Cy3 versus Cy5 of LMB at variable pH from 7 to 3. 3A reproduced by the permission from ref 17. Copyright 2013 Springer Nature Limited. 3B-D reproduced with permission from ref 5. Copyright 2016 The Royal Society of Chemistry.

for DNA-based nanomachines designed for interrogation of cellular pathways or cargoes delivery. Krishnan et al. reported DNA nanomachines to simultaneously map the pH changes in two distinct cellular environments inside a living cell.[17] The two nanomachines with chosen FRET pairs having minimal cross-talk were coupled to interrogate pH along the distinct organellar pathways. The FRET-based nanomachines were programmed to deliver them to a different endosomal pathway. The pH-sensitive regimes of the two DNA nanomachines were tailored for the lumenal pH of the relevant intracellular organelles. The furin retrograde endocytic pathway and the transferrin recycling pathway were selected for interrogation, which lead to the trans-Golgi network and perinuclear recycling endosomes, respectively. Especially, a molecularly programmable DNA nanomachine called I^{Fu} was employed to trace the furin pathway. The nanomachine was optimized with an extra eight base pair sequence that can be used for the conjugation of the protein that localizes the nanodevice and targeted to the transferrin pathway via receptor mediated endocytosis. The authors have developed a pH-sensitive double-stranded DNA nanomachine to study the transferrin pathway. This nanomachine I^{Tf} (transferrin pathway) consists of a pH-responsive C-rich segment (depicted in purple) which forms a weak duplex through partial base pairing with a G-rich overhang in the complementary region. The duplex formation permits the C-rich region to fold into an intramolecular *i*-motif at lower pH levels (Figure 3A). The chemical conjugation of transferrin to one of the DNA strands allowed the molecular programmability to confine I^{Tf} endocytosis via transferrin receptor pathway. The nanomachines developed were found to be effective in the pH range of 5 to 6.5 and can be used to investigate typical intraorganellar pH. The cellular studies using Hela cells showed the internalization and localization of nanodevices to the target compartments.

Intracellular pH homeostasis is crucial to cellular health and controls a variety of biological functions. In this context, our group has developed a reversible molecular beacon (MB) to A-motif transformation-based molecular DNA architecture for monitoring of intracellular acidic conditions in the range of pH 3 to 5.[5] A DNA nanoswitch was designed wherein A-rich MB transforms from a closed state to an open state (A-motif) under acidic pH conditions. The MB DNA sequence consists of 24 base pairs adapts a hairpin-like structure (unlabeled MB: UMB, 5′-GACGCCAAAAAAAAAAACGCGTC-3′, and labeled MB: LMB, Cy3−5′-GACGCCAAAAAAAAAAACGCGTC-3′-Cy5) (Figure 3B,C). The hairpin-like duplex structure is formed by two stretches of 5 complementary base pairs each at 5′- and 3′-ends and 12 consecutive As form the loop region (UMB). The 5′ and 3′ ends of MB (LMB) are labeled with Cy3 and Cy5 dyes, respectively. Cy3 and Cy5 dyes were chosen as the FRET pair due to high fluorescence quantum yield, effective FRET response, and fluorescence insensitivity to acidic conditions (pH ∼ 3). LMB forms a hairpin-like structure (closed state) under neutral pH conditions, while in acidic conditions, it transforms into an open-state (A motif) via reverse Hoogsteen [AH^+-H^+A] hydrogen-bonding interactions attributed to protonation of As. Further, the electrostatic interactions between the AH^+ and the phosphate backbone stabilize A-motif, resulting in the reduction of FRET efficiency. (Figure 3D). Changing from pH 7 to 3 and vice versa, the DNA nanoswitch was found reversibly switching between MB and A-motif states (Figure 3E). Furthermore, the DNA nanoswitch was tested in an artificial vesicle that mimics the cell-like environment. Cy3 and Cy5-labeled LMB was encapsulated into the vesicles under the neutral pH condition. An excellent FRET signal was observed in the pH window of 5 to 3 using fluorescence microscopy and sensitivity of the change in FRET signal capable of detecting small pH changes with step sizes of 0.2−0.4 units. Facile cellular delivery of the short oligonucleotide-based LMB at pH 7.4 was demonstrated in HeLa cells, without the use of an external transfection agent. The ratio of change in FRET between Cy3 and Cy5 was used to monitor the successful transformation of LMB from the closed state to the open state inside live HeLa cells. This provides the real-time, rapid, and sensitive sensing of change in intracellular acidic pH.

Shi et al. used GQ DNA and a ruthenium(II) complex (Ru) to construct a molecular "light switch" based on a pH-controlled on−off−on mechanism.[18] A polymorphic quadruplex can be formed by the G-rich DNA sequence consisting

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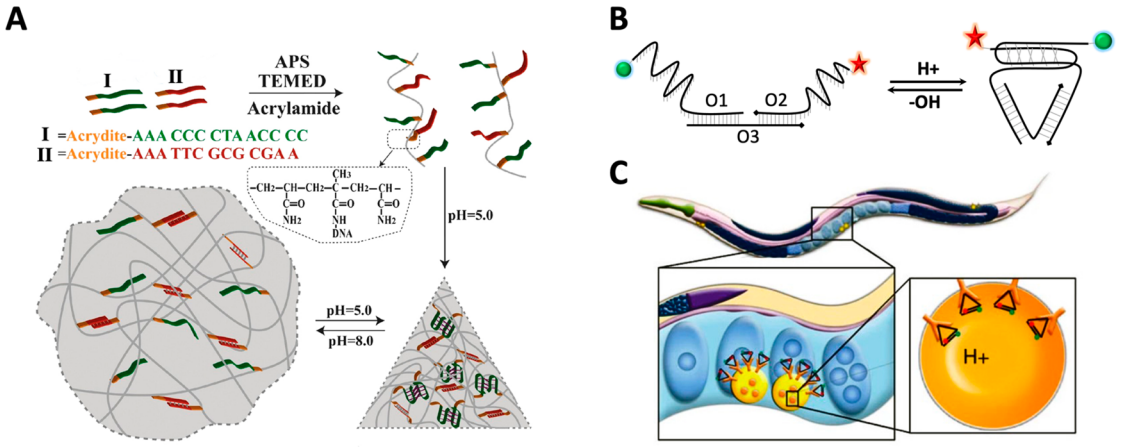

Figure 4. (A) Stimuli-responsive switchable nucleic acid (I) and (II)-functionalized acrylamide copolymer hydrogel structure and its quasi-liquid state. (B) Structure and working principle of I-switch as DNA nanomachine. (C) I-switch uptake in coelomocytes postinjection in *C. elegans*. Panel A reproduced from ref 20. Copyright 2014 John Wiley & Sons. Panels B and C reproduced by the permission from ref 21. Copyright 2011 Springer Nature Limited.

of stacked coplanar G-tetrads supported by Hoogsteen hydrogen bonds. In the presence of K^+, the G-rich DNA sequence forms a hybrid-type mixed parallel/antiparallel GQ structure. In detail, an imidazole moiety present in the main ligand of Ru-complex showed enhancement in fluorescence intensity in the presence of GQ DNA, which is visibly detected by the naked eye. The Ru complex is nonemissive in acidic pH below 1.4, which corresponds to the "off" state. Upon the increase of pH to 4.5 by the addition of KOH, the complex exhibits enhanced emission intensity, which is considered as the "on" state. A combination of fluorescence and circular dichroism (CD) spectroscopy techniques was used to validate the switching mechanism. The GQ conformational switch transformed from a hybrid structure to an antiparallel structure over a pH range of 4.5 to 2.5. Attaining pH 1.4 leads to the quenching of fluorescence, which implies the collapse of the antiparallel structure to ssDNA and that the Ru complex is no longer bound with ssDNA. Thus, a reversible pH-modulated dual switch analogy was developed to detect a conformational change of GQ by a "light switch" approach. Overall, programming nucleic acid architectures for reversible or switchable transformation between distinct conformational states in response to stimuli are anticipated to find applications in the field of imaging and diagnosis of diseases that are characterized by marked changes in pH like lysosomal storage disorder.[17]

pH-RESPONSIVE DNA-BASED HYDROGEL

The development of stimuli-responsive scaffolds is a rapidly growing area in the field of DNA molecular and nanoarchitectonics. The stimuli-triggered or stimuli-controlled hydrogel transforms to a solution or exhibits a change in stiffness, which attracts a variety of applications requiring transformable mechanical properties. Liu and co-workers reported hydrogel of a three-armed (Y-shaped) DNA nanostructural unit based on the *i*-motif induced molecular interlocking and hydrogel formation.[19] The Y-shaped DNA structural units in *β*-morpholinoethanesulfonic acid (MES) media do not interact under physiological conditions because of electrostatic repulsion; however, upon reducing the pH to 5.0, the intermolecular *i*-motif was formed within a minute, which results in three-dimensional cross-linking of structural units driving the solution to a gel state. The partial protonation of Cs at low pH (pH 5), resulting in the formation of $C{\cdot}CH^+$ through triple hydrogen bonding interaction between protonated (CH^+) and unprotonated Cs from the underlying molecular glue to cross-link the Y-shaped units. The *i*-motif domains inside the same Y unit are linked to the rigid double-stranded central domains and point in different directions in the design, preventing intra-Y-unit *i*-motifs formation. Therefore, this design strategy favors inter-Y-unit *i*-motif structure formation which accumulates to form DNA hydrogel. Later, the same group reported a DNA motor based on pH-dependent conformational changes from the *i*-motif to single-strand transformation to tune the mechanical property of DNA hydrogel. The pH-triggered reversible opening of the *i*-motif's conformation relaxes the distance between distinct cross-linking locations, which determines the stiffness of the DNA hydrogel. The controlled modulation of mechanical strength has a beneficial implication on stem cell differentiation and tissue engineering applications.

The precise stimuli-responsive modulation of mechanical properties can be used to formulate hydrogels with a reversible change in stiffness or shape, which is referred to as the shape-memory property. Willner and co-workers designed a pH-responsive DNA hydrogel with the shape-memory property. DNA hydrogel can be processed into a permanent shape and programmable to take up a temporary shape that stores the code to restore to the original shape at an appropriate pH. The molecular design involves an acrylamide copolymer chain incorporated with a duplex (I) and *i*-motif (II) forming DNA sequences that form a hydrogel. In this system, the acrydite-modified with DNA I and II were copolymerized with acrylamide residues. DNA I that consists of a C-rich sequence is the precursor for *i*-motif architecture, while DNA II consists of a self-complementary sequence. A triangle-shaped mold was used to make the hydrogel of a defined shape, which was then removed. At pH 5.0, a stable triangle-shaped hydrogel is formed because acrylamide the cross-linking copolymer produces an *i*-motif structure of DNA I and duplexes containing DNA II. Eventually, a pH-responsive switchable triangle-shaped hydrogel transforms to an amorphous state at pH 8.0, and reacidifying the hydrogel to pH 5.0 restores the triangle-shaped structure (Figure 4A).[20] This study introduced

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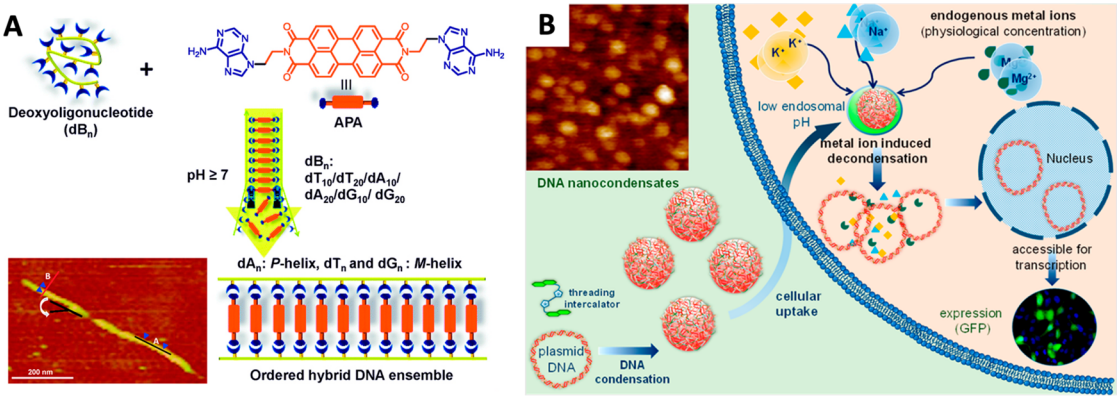

Figure 5. (A) Double-zipper helical assembly of **APA** with different oligonucleotides and high resolution AFM image of $[dT_{20}:(\mathbf{APA})_{20}:dT_{20}]$. (B) Threading intercalator induced DNA condensation, decondensation, cellular uptake, pH-dependent tracking, metal ion induced DNA release and delivery. Panel A reproduced with permission from ref 22. Copyright 2015 The Royal Society of Chemistry. Panel B reproduced with permission from ref 24. Copyright 2020 American Chemical Society.

stimuli-responsive switchable DNA-based hydrogels with shape-memory capabilities, by insertion of two functional cross-linking copolymers into a hydrogel which have potential application in new sensors, drug-delivery matrices, information-encoding materials, and selective cell adhesion matrices.

DNA-TARGETED BIOIMAGING

Probing intracellular conditions such as cation concentrations and pH in eukaryotic cells is essential to understanding various physiological processes. A DNA nanomachine (I-switch) was developed to measure pH spatiotemporally in live cells.[21] The molecular design of the I-switch consisted of two ssDNA sequences (O1 and O2) partly WC hydrogen bonded to a flanking sequence in O3 separated by a single nucleotide hinge (Figure 4B). The unhybridized free part of O1 and O2 are C-rich and conjugated to donor and acceptor fluorophores Alexa-488 and Alexa-647 on O1 and O2, respectively. Upon lowering the pH, the C-rich regions are protonated, and two parallel-stranded C-H·C^+ bonding interactions facilitate the *i*-motif formation, concurrently bringing the donor and acceptor fluorophores to close proximity which results in FRET. The I-switch was employed to measure pH along the endocytic pathway in vivo in *C. elegans*. The pseudocoelomic cavity of *C. elegans* has six scavenger cells called coelomocytes that continuously endocytose macromolecules from the body cavity, thus forming an interesting organism to study pH heterogeneity. The endocytic uptake of I-switch is mediated by receptors in the coelomocytes called anionic ligand-binding receptor (ALBR), which was validated by competitive binding experiments using ligands known to bind ALBRs and mutated hermaphrodites with compromised ALBR receptors. The coelomocytes at two pH regimes (pH 5 and 7) showed a remarkable correlation of pH-dependent labeling intensity of the I-switch, as observed by the in vivo quantification. The emission intensity of the endocytosed I-switch in the intermediate pH range ($5 \leq pH \leq 7$) was measured, which helped in the spatiotemporal correlation of the I-switch transport along the early endosome, late endosome, and lysosomes in coelomocytes of *C. elegans* (Figure 4C). The I-switch has a response time of 1−2 min, which allows reporting of fine spatial and temporal pH changes associated with biological processes. In general, there are a lot of possibilities for employing DNA nanoarchitectures and devices to probe various biological processes of interest in whole organisms.

DNA ARCHITECTONICS-GUIDED DRUG DELIVERY

DNA nanoarchitectures are potential carriers to deliver drugs and bioactive ingredients due to their molecular programmability, ease of structural modification, and spatiotemporal addressability. A novel concept of the small functional molecule (SFM)-templated DNA nanotechnology or functional DNA nanoarchitectonics has been developed by our group. This involves templated and mutual assembly of short oligonucleotides and SFM to create functional DNA architectures following the scheme of molecular architectonics.[1,3−5,23] In an elegant design, symmetrically functionalized perylene bisimide (PBI) with As (**APA**) was employed as a universal SFM template to construct hybrid DNA ensembles. A form WC hydrogen bonding with T and non-WC with all other nucleobases and this intriguing property of A was motivated us to design a double zipper template **APA**. Because of excellent molecular and electronic properties, PBI finds use in a wide variety of optoelectronics and biomedical applications. The WC interaction between the A of **APA** and the complementary T of dT_n, supported by the appropriately adjusted hydrophobic forces and $\pi-\pi$ stacking integrations of perylene core led to the formation of **APA** assembly templated hybrid DNA ensemble. The CD spectroscopy and high-resolution atomic force microscopy (AFM) studies of **APA** and dT_{20} have revealed the formation of a mutually templated chiral ensemble of the type $[dT_{10}:(\mathbf{APA})_{10}:dT_{10}]$ and $[dT_{20}:(\mathbf{APA})_{20}:dT_{20}]$ with M-helicity (left handed helix) (Figure 5A). However, $[dA_{10}:(\mathbf{APA})_{10}:dA_{10}]$ and $[dG_{10}:(\mathbf{APA})_{10}:dG_{10}]$ revealed the formation of P- and M-helical ensembles, respectively. Further, the pH-responsive "double zipper" assembly of **APA** and oligonucleotides collapse in the acidic pH environment because of disruption of hydrogen bonding, which can be used for pH-dependent drug delivery system for small molecules and oligonucleotides.[22]

Recently, we have developed a threading intercalator mediated nanocondensation of DNA for its effective cellular uptake, spatiotemporal tracking, pH and metal ion triggered release and transfection. DNA condensation forms an essential prerequisite for transfection, wherein packaging of large DNA like plasmids into condensates with reduced surface negative charge increases cellular uptake and avoids degradation by nucleases. The design of the ligands is based on molecular scaffolds that can bind to DNA through unique intercalation and groove binding mechanisms (which is termed threading

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intercalation) with minimal electrostatic interactions to reduce cellular toxicity. A designed bis-imidazolium ligand is composed of two imidazolium rings connected by an ethyl-bridge and linked to aromatic moieties such as naphthyl or quinolinyl via amide or ether linkages. The bis-imidazolium ligand threads across the groove, while the aromatic moieties appended at either end of the linker intercalates between the nucleobases in DNA. The cumulative threading intercalation of the ligand and DNA results in the formation of nano-architectures of an average size of 100 nm as visualized by AFM. Specifically, the bis(imidazolium-hydroxy-quinolinyl) (BIHQ) ligand was found to be non-toxic and the most viable candidate for DNA condensation to form nanocondensates. The fluorescence emission of quinoline moiety in acidic pH was used to track the delivery and release of DNA nanocondensates in the endocytic pathway. The quinolinyl moiety of BIHQ remains unprotonated at the normal physiological (pH ∼ 7.2) conditions. In acidic conditions (pK_a ∼ 4−5) of the endocytic pathway, the quinolinyl moiety undergoes protonation and exhibit fluorescence (Figure 5B).[24] The pH decreases gradually in the endocytic pathway from ∼6.3 in early endosomes, ∼5.5 in late endosomes to ∼4.5 in lysosomes. The colocalization of fluorescently tagged DNA and BIHQ in cellulo proved the successful intracellular delivery of the DNA nanocondensates. The endogenous metal ions such as Mg^{2+} and K^+ were found to induce DNA decondensation at their endogenous concentrations providing a viable mechanism of DNA decondensation within cells. The proof-of-concept study in HEK 293T cells established the threading-intercalator-based molecular platform for condensation, cellular uptake, pH-responsive in cellulo tracking of nanocondensates, and metal-ion-mediated release of DNA, and transfection.

Linko et al. designed a DNA nanocapsule equipped with rationally designed pH-latches.[25] The latches consist of dsDNA and ssDNA, which either form a parallel triplex at a lower pH (6.3) or free overhangs at a higher pH (7.7) range, resulting in the closed and open state of the nanocapsule, respectively. The programmability of capsule is monitored by FRET measurements. The capsule is inbuilt with a functionalizable cavity for the encapsulation of a molecular payload. When the pH is elevated, the capsule opens rapidly releasing the encapsulated payload. This study showed that the nanocapsule can be loaded with various types of molecular cargo like gold nanoparticles and horseradish peroxidase, and cargoes can be selectively released after exposing to the pH stimuli. This type of pH-responsive DNA-based carrier without the requirement of any external triggers is a promising strategy for controlled drug binding and delivery. The ultimate goal of nucleic acid architectonics is to construct novel architectures of DNA/RNA and SFMs with emerging features, properties, and applications.

CONCLUSION AND OUTLOOK

In summary, we discussed recent advancements in nucleic acid architectonics to construct molecular and material architectures based on pH-responsive DNA systems and their multifarious applications. This emerging field has re-emphasized the role of DNA as an important building block in material science. The scheme of molecular architectonics guide the development of functional DNA/RNA nanoarchitectures with potential impact in nanoscience and nanotechnology. High-fidelity interactions among nucleobases and predictability over the architectural dynamics in response to external stimuli like pH makes DNA/RNA as the material building block of choice for the design of nanosystems and devices. The canonical (WC) and noncanonical (non-WC) hydrogen bonding, hydrophobic, aromatic $\pi-\pi$ stacking, van der Waals, electrostatic, and metal-based interactions play a crucial role in the construction of nucleic acid nanoarchitectures that are responsive to exogenous or endogenous stimuli such as pH. The molecular architectonics-guided templated assembly of oligoncelotides (DNA/RNA) and small functional molecules hold great promise in generating functional nanoarchitectures with practical applications. The external stimuli serve as a trigger to affect the reversible conformational or structural changes of canonical and noncanonical structures of nucleic acids (DNA/RNA), which is the guiding principle for the development of stimuli responsive smart molecular architectures and devices. These nucleic acid-based architectures and devices are promising tools for multifarious material and biomedical applications, ranging from (bio)sensing, (bio)-electronics, diagnostics, and drug delivery to therapy.

The extraordinary features including programmability, biocompatibility, and ease of structural and functional tunability, makes nucleic acids a desired material building blocks for the construction of smart molecular architectures and devices with the potential for materials technologies to biomaterial applications. The challenges that need to be addressed in the field of nucleic acid architectonics include development of reliable and controllable DNA systems and devices through coassembly and templated assembly of nucleic acids mutually templated with small functional molecules for practical applications. Further, design of multistimuli-responsive nucleic acid nanoarchitectures with unprecedented applications in the interrelated-domains of health, energy, and environment are of utmost importance.

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Notes

The authors declare no competing financial interest.

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