Nucleic Acid Architectonics for pH-Responsive DNA Systems and Devices

Mohamed Nabeel Mattath, Debasis Ghosh, Sumon Pratihar, Shuo Shi, and Thimmaiah Govindaraju*

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. 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 sp2-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). The ability of A to form A:T, A:A, and A:G pairs has inspired the development of hybrid DNA ensembles. 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). 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.

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 replacement of classic WC base pairing interaction with metal complexation is another important development and metal base pairing interaction potentially generates DNA-templated nanoarchitectures. 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 (Hg²⁺) binds to T to form a T-Hg²⁺-T complex (Figure 1G). 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⁺. 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.

The expansion of DNA nanotechnology has allowed development of high-fidelity programmable systems that can perform various functions and are of immense importance in the fields of diagnostics and therapeutics. The pH-responsive functional DNA systems and devices offer several advantages such as biocompatibility, structural programmability, and stability under physiological conditions. 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.
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 N3 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), the fluorophore moves away from the quencher resulting in an enhanced fluorescence signal. The fluorescence signal reveals the formation of a duplex structure, while

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 small molecules | diverse secondary and tertiary conformations                        | highly unstable, expensive, and sensitive to RNases                  |
| peptides/ proteins | high potency and selectivity, high chemical and biological diversity, ease of synthesis and broad tunability, and pH-responsive nature is reasonably established | unique properties and pH dependency of small molecules cannot be generalized |
| 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 | poor metabolic stability, weak membrane permeability, low oral bioavailability and predictable behavior toward pH |
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 (dBn, 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. Because 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

---

**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.
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 pH 8.0. 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 access 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-nanoclusters (DNA-AgNCs) exploiting the strong a and G15H because of the strand displacement at pH 5.0, addition of miR-155 causes repetitive hairpin assembly of HB fl motif tetraplex in two hairpin probes. In PBS bu identical C-rich sequences are introduced for one-half of the expression in cancer, miR-155 was selected as a target, and

conformational change bringing the G₃ 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 (dc₁₂) based DNA-AgNCs for sensing Fe(III) selectively among other metal ions. The dc₁₂ 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
Intracellular pH homeostasis is crucial to cellular health and 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. A polymorphic quadruplex can be formed by the G-rich DNA sequence consisting 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. 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 tailored IFu endocytosis via transferrin pathway 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. 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, without the use of an external transfection agent. 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.

Krishnan et al. reported for DNA-based nanomachines designed for interrogation of cellular pathways or cargoes delivery. For DNA-based nanomachines designed for interrogation of cellular pathways or cargoes delivery.
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 structural unit based on the i-motif architecture, while DNA II consists of a C-rich sequence and is functionalized 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 is a molecular glue to cross-link the Y-shaped units. 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 is a molecular glue to cross-link the Y-shaped units. 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 is a molecular glue to cross-link the Y-shaped units. 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.
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. 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 pseudozoelocimic 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 ≤ pH ≤ 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 nanorobots 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. In an elegant design, symmetrically functionalyzed perylene bisimide (PBI) with As (AP) 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 dT10 supported by the appropriately adjusted hydrophobic forces and π-π 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 dT20 have revealed the formation of a mutually templated chiral ensemble of the type [dT10(A)10:dT10] and [dT20:APA5:dT20] with M-helicity (left handed helix) (Figure S5A). However, [dA10(A)10:dA10] and [dG10:APA10:dG10] 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.

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

**Figure 5.** (A) Double-zipper helical assembly of APA with different oligonucleotides and high resolution AFM image of [dT20:APA10: dT10]. (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.
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 nanoarchitectures of an average size of 100 nm as visualized by AFM. Specifically, the bisimidazolium-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 the ligand and DNA results in the formation of nanocondensates (Figure 5B).24

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 π–π 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 oligonucleotides (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.

**AUTHOR INFORMATION**

Corresponding Author
Thimmiaiah Govindaraju – Bioorganic Chemistry Laboratory, New Chemistry Unit and School of Advanced Materials (SAMat), Jawaharlal Nehru Centre for Advanced Scientific Research, Bengaluru, Karnataka 560064, India; orcid.org/0000-0002-9423-4275; Email: tgraju@jncsr.ac.in

Authors
Mohamed Nabeel Mattath – Bioorganic Chemistry Laboratory, New Chemistry Unit and School of Advanced Materials (SAMat), Jawaharlal Nehru Centre for Advanced Scientific Research, Bengaluru, Karnataka 560064, India; School of Chemical Science and Engineering, Tongji University, Shanghai 200092, PR China
Debasis Ghosh – Bioorganic Chemistry Laboratory, New Chemistry Unit and School of Advanced Materials (SAMat), Jawaharlal Nehru Centre for Advanced Scientific Research, Bengaluru, Karnataka 560064, India
Sumon Pratihar – Bioorganic Chemistry Laboratory, New Chemistry Unit and School of Advanced Materials (SAMat), Jawaharlal Nehru Centre for Advanced Scientific Research, Bengaluru, Karnataka 560064, India
Shuo Shi – School of Chemical Science and Engineering, Tongji University, Shanghai 200092, PR China; orcid.org/0000-0003-4387-3445

Complete contact information is available at:
Notes
The authors declare no competing financial interest.

Biographies
Mohamed Nabeel Mattath received his M.Sc. in Chemistry from Jamal Mohamed College affiliated to Bharathidasan University, Tamil Nadu, India in 2016. He obtained his M.Phil. in Inorganic Chemistry from the University of Madras, Tamil Nadu, India in 2018. He is pursuing a Ph.D. under the supervision of Professor Shuo Shi at the School of Chemical Science and Engineering, Tongji University, Shanghai, China. Currently, he is working as a visiting student with Prof. T. Govindaraju at Bioorganic Chemistry Laboratory, JNCASR, India. His research interest includes designing DNA-based molecular devices, DNA nanotechnology, biosensing, and biocomputing applications.

Debasis Ghosh received his BSc (Hons.) in Chemistry from Midnapore College, West Bengal, India in 2013, and MSc in Chemistry from Indian Institute of Technology Guwahati in 2015. Currently, he is pursuing a Ph.D. under the supervision of Prof. T. Govindaraju, at JNCASR. His research interests include design and synthesis of peptides, modulation of protein aggregation, understanding the structural aspects of peptidomimetics by utilizing various biophysical approaches, and nanostructured materials.

Sumon Pratihar received his BSc (Hons.) in Chemistry from Midnapore College, West Bengal, India in 2013, and MSc in Chemistry from Indian Institute of Technology Madras in 2015. Currently, he is pursuing a Ph.D. under the supervision of Prof. T. Govindaraju, at JNCASR. His research interests are in understanding and employing the interaction of synthetic organic molecules with nucleic acids for diagnostic and therapeutic applications.

Shuo Shi is a full professor in the school of Chemical Science and Engineering, Tongji University. He received his Ph.D. in chemical biology from Sun Yat-Sen University (SYSU) in 2007. From 2012 to 2013, he worked as a visiting scientist at the California Institute of Technology (Caltech). He joined Tongji University in 2007, and his research interests include DNA-based biosensors/devices, metal-based anticancer drugs, nanomaterials for tumor accurate diagnosis, and synergistic therapy. Currently, he is pursuing a Ph.D. under the supervision of Prof. T. Govindaraju, at JNCASR. His research interests include design and synthesis of DNA nanomachines, and DNA-based bisensoric and synergistic therapy.

T. Govindaraju is a Professor at Bioorganic Chemistry Laboratory, New Chemistry Unit, JNCASR, Bengaluru, India. He received his MSc in Chemistry (2000) from Bangalore University and Ph.D. (2005) from National Chemical Laboratory and University of Pune, India. He carried out postdoctoral research (2005–2006) at the University of Wisconsin-Madison, United States. He received the Alexander von Humboldt postdoctoral fellowship (2006–2008) and worked in the Max Planck Institute of Molecular Physiology, Dortmund, Germany. His research interests are at the interface of chemistry, biology, and biomaterials science, including Alzheimer’s disease, molecular probes, diagnostic therapy (theranostics), molecular architectonics, and silk and cyclic dipeptide-derived biomaterials.

Acknowledgments
Authors thank Prof. C. N. R. Rao FRS for his constant support and encouragement, JNCASR, the DST-Nanomission (grant: DST/SMS/4428 or SR/NM/TP-25/2016), DST-Swarnajayanti Fellowship, Government of India, and Sheikh Saqr Laboratory (SSL), ICMS-JNCASR for financial support, CSC, Chinese Government Scholarship to M.N.M., China, and UGC fellowship to S.P.

References
(1) Govindaraju, T. Templated DNA Nanotechnology: Functional DNA Nanoarchitectonics; CRC Press, 2019.
(2) Roy, B.; Ramesh, M.; Govindaraju, T. DNA-Based Nanoswitches and Devices. In Templated DNA Nanotechnology; Jenny Stanford Publishing, 2019, pp 365–408.
(3) Ghosh, D.; Datta, L. P.; Govindaraju, T. Molecular architectonics of DNA for functional nanoarchitectures. Beilstein J. Nanotechnol. 2020, 11 (1), 124–140.
(4) Pandeeswar, M.; Senanayak, S. P.; Govindaraju, T. Nanoarchitectonics of small molecule and DNA for ultrasensitive detection of mercury. ACS Appl. Mater. Interfaces 2016, 8 (44), 30362–30371.
(5) Narayananswamy, N.; Nair, R. R.; Suseela, Y.; Saini, D. K.; Govindaraju, T. A molecular beacon-based DNA switch for reversible pH sensing in vesicles and live cells. Chem. Commun. 2016, 52 (56), 8741–8744.
(6) Avinash, M.; Govindaraju, T. Architectonics: design of molecular architecture for functional applications. Acc. Chem. Res. 2018, 51 (2), 414–426.
(7) Moorthy, H.; Datta, L. P.; Govindaraju, T. Molecular Architectonics-guided Design of Biomaterials. Chem. Asian J. 2021, 16 (5), 423–442.
(8) (Molecular Architectonics and Nanoarchitectonics; Springer Nature Series of Nanostructure Science and Technology; Govindaraju, T., Ariga, K., Eds.; Springer Nature: Singapore, 2021.
(9) Idili, A.; Vallée-Belisle, A.; Ricci, F. Programmable pH-triggered DNA nanoswitches. J. Am. Chem. Soc. 2014, 136 (16), 5836–5839.
(10) Liu, D.; Balasubramanian, S. A proton-fuelled DNA nanomachine. Angew. Chem., Int. Ed. 2003, 42 (46), 5734–5736.
(11) Yang, S.; Liu, W.; Wang, R. Control of the stepwise assembly-disassembly of DNA origami nanoclusters by pH stimuli-responsive DNA triplexes. Nanoscale 2019, 11 (39), 18026–18030.
(12) Williamson, P.; Ijäs, H.; Shen, B.; Corrigan, D. K.; Linko, V. Probing the conformational states of a pH-sensitive DNA origami zipper via label-free electrochemical methods. Langmuir 2021, 37 (25), 7801–7809.
(13) Kim, S. H.; Kim, K.-R.; Ahn, D.-R.; Lee, J. E.; Yang, E. G.; Kim, S. Y. Reversible regulation of enzyme activity by pH-responsive encapsulation in DNA nanocages. ACS Nano 2017, 11 (9), 9352–9359.
(14) Li, C.; Chen, Z.; Zhang, Y.; He, J.; Yuan, R.; Xu, W. Guanine-lighting-up fluorescence biosensing of silver nanoclusters populated in functional DNA constructs by a pH-triggered switch. Anal. Chem. 2020, 92 (19), 13369–13377.
(15) Guo, L.; Tang, T.; Hu, L.; Yang, M.; Chen, X. Fluorescence assay of Fe (III) in human serum samples based on pH dependent silver nanoclusters. Sens. Actuators B Chem. 2017, 241, 775–778.
(16) Patino, T.; Porchetta, A.; Jannasch, A.; Lladó, A.; Stumpf, T.; Schäffer, E.; Ricci, F.; Sánchez, S. Self-sensing enzyme-powered micromotors equipped with pH-responsive DNA nanoswitches. Nano Lett. 2019, 19 (6), 3440–3447.
(17) Modi, S.; Nizak, C.; Surana, S.; Halder, S.; Krishnan, Y. Two DNA nanomachines map pH changes along intersecting endocytic pathways inside the same cell. Nat. Nanotechnol. 2013, 8 (6), 459–467.
(18) Yao, J.-L.; Gao, X.; Sun, W.; Fan, X.-Z.; Shi, S.; Yao, T.-M. A naked-eye on-off-on molecular “light switch” based on a reversible “conformational switch” of G-quadruplex DNA. Inorg. Chem. 2012, 51 (23), 12591–12593.
(19) Cheng, E.; Xing, Y.; Chen, P.; Yang, Y.; Sun, Y.; Zhou, D.; Xu, L.; Fan, Q.; Liu, D. A pH-triggered, fast-responsive DNA hydrogel. Angew. Chem., Int. Ed. 2009, 48 (41), 7660–7663.
(20) Guo, W.; Lu, C. H.; Orbach, R.; Wang, F.; Qi, X. J.; Cecconello, A.; Seliktar, D.; Willner, I. pH-Stimulated DNA Hydrogels Exhibiting Shape-Memory Properties. Adv. Mater. 2015, 27 (1), 73–78.
(21) Surana, S.; Bhat, J. M.; Koushika, S. P.; Krishnan, Y. An autonomous DNA nanomachine maps spatiotemporal pH changes in a multicellular living organism. Nat. Commun. 2011, 2 (1), 340.
(22) Narayanaswamy, N.; Suresh, G.; Priyakumar, U. D.; Govindaraju, T. Double zipper helical assembly of deoxyoligonucleotides: mutual templating and chiral imprinting to form hybrid DNA ensembles. *Chem. Commun.* 2015, *51* (25), 5493−5496.

(23) Narayanaswamy, N.; Avinash, M. B.; Govindaraju, T. Exploring hydrogen bonding and weak aromatic interactions induced assembly of adenine and thymine functionalised naphthalenediimides. *New J. Chem.* 2013, *37* (5), 1302−1306.

(24) Pratihar, S.; Suseela, Y. V.; Govindaraju, T. Threading Intercalator-Induced Nanocondensates and Role of Endogenous Metal Ions in Decondensation for DNA Delivery. *ACS Appl. Bio Mater.* 2020, *3* (10), 6979−6991.

(25) Ijäs, H.; Hakaste, I.; Shen, B.; Kostiainen, M. A.; Linko, V. Reconfigurable DNA origami nanocapsule for pH-controlled encapsulation and display of cargo. *ACS Nano* 2019, *13* (5), 5959−5967.