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INVITED REVIEW

Nucleic acid secondary structures for therapeutic and biomolecular device applications

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Abstract

Nucleic acids can form a variety of non-canonical secondary structures, among which G-quadruplexes and i-motifs are well-studied. These non-canonical structures are widespread in the human genome and are primarily found in the telomeric region and the promoter region of numerous proto-oncogenes. Hence, they represent attractive pharmacological targets for anti-cancer therapeutics. Besides, the unique properties of these structures can also be utilized for nano-biotechnology applications. This review highlights the therapeutic targeting of G-quadruplexes and i-motifs by selective ligands endowed with potent biological activities. These novel molecular probes can selectively target DNA secondary structures over duplex DNA and promote cancer cell death. We also outline the development of advanced functional nanostructures like ion channels, bio-nanowires, logic gates, and enzyme-regulated DNA-based devices by conjugating G-quadruplex or i-motif with organic scaffolds. This review also focuses on the supramolecular chemistry of nucleic acid components to generate nucleoside-derived hydrogel networks and synthetic ion channels. These studies would provide new dimensions into anti-cancer therapeutics and nano-biotechnology using DNA secondary structures.

Keywords: anti-cancer therapeutics; biomolecular devices; gene regulation; G-quadruplex; i-motif; ligands

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Dr. Jyotirmayee Dash is a professor in the School of Chemical Sciences at the Indian Association for the Cultivation of Science (IACS), Kolkata. She obtained her Ph.D. in Organic Chemistry from IIT Kanpur, India, under the guidance of Professor F. A. Khan. She earned the Alexander von Humboldt fellowship with Professor H.-U. Reissig at the Freie University of Berlin and a postdoctoral fellowship with Professor J. Cossy at ESPCI Paris. She has also been awarded a Marie Curie fellowship with Professor S. Balasubramanian at the University of Cambridge, UK. She became an Assistant Professor at IISER-Kolkata from 2009-2012. In 2012, she joined IACS. She subsequently received DST Swarnajayanti Fellowship, DBT Wellcome Trust Fellowships, and Shanti Swarup Bhatnagar Prize. Her research mainly focuses on small molecule mediated regulation of gene expression and the structure and function of nucleic acids and their components.

Introduction

Nucleic acids are the information-carrying molecules of the cell and form the basis for the transmission of genetic traits from one generation to another. They are composed of phosphoric acid, sugars, and a mixture of organic bases (purines and pyrimidines). The name "nucleic acid" is derived from the fact that they represent a significant constituent of the cell nucleus and their acidic properties. The two main classes of nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA is the master blueprint for life and forms the genetic material for all free-living organisms and most viruses. RNA is the genetic material of certain viruses, but it is also found in all living cells, where it plays an essential role in specific processes such as protein synthesis.

The DNA structure was first postulated in 1953 by Watson and Crick, which remains a ground-breaking discovery in modern genetics. They proposed a right-handed double-helical model for the structure of DNA.[1] However, DNA is also capable of forming other structures. It later became clear that DNA has enormous ability to adopt various alternative conformations like G-quadruplexes, i-motifs, etc. which play essential roles in nucleic acid targeted therapeutics.[2,3]

G-quadruplex and i-motif

G-quadruplex (G4) and i-motif (iM) are four-stranded non-canonical nucleic acid secondary structures formed from guanine (G)-rich and cytosine (C)-rich sequences, respectively. Recent evidences suggest that these DNA structures exist in living cells.[4,5] They could also be involved in several cancer-related processes, thus representing attractive targets for anti-cancer drug discovery.[6-8]

G-quadruplexes are formed from G-rich sequences and consist of stacked planar guanosine tetrads (G-tetrads or G-quartets). Each of the quartets arises from the planar association of four guanine molecules by Hoogsteen hydrogen-bonding.[9] The stability of the G-quadruplex structure largely depends on monovalent cations, specifically K^+ and Na^+ (Figure 1). These structures are also stable under physiological conditions.[10] Recent studies using G4-specific antibodies[5] and *in vivo* NMR[11] suggested their *in vivo* existence. Most of these G4 forming sequences are prevalent within the genome's regulatory regions, particularly within the promoter region of various genes (*c-MYC, VEGF, BCL-2, KRAS*, *c-KIT, etc.*) and in the telomeres, suggesting their role in gene transcription.[12-19] Guanine-rich RNA sequences can also form stable G- quadruplexes, which are

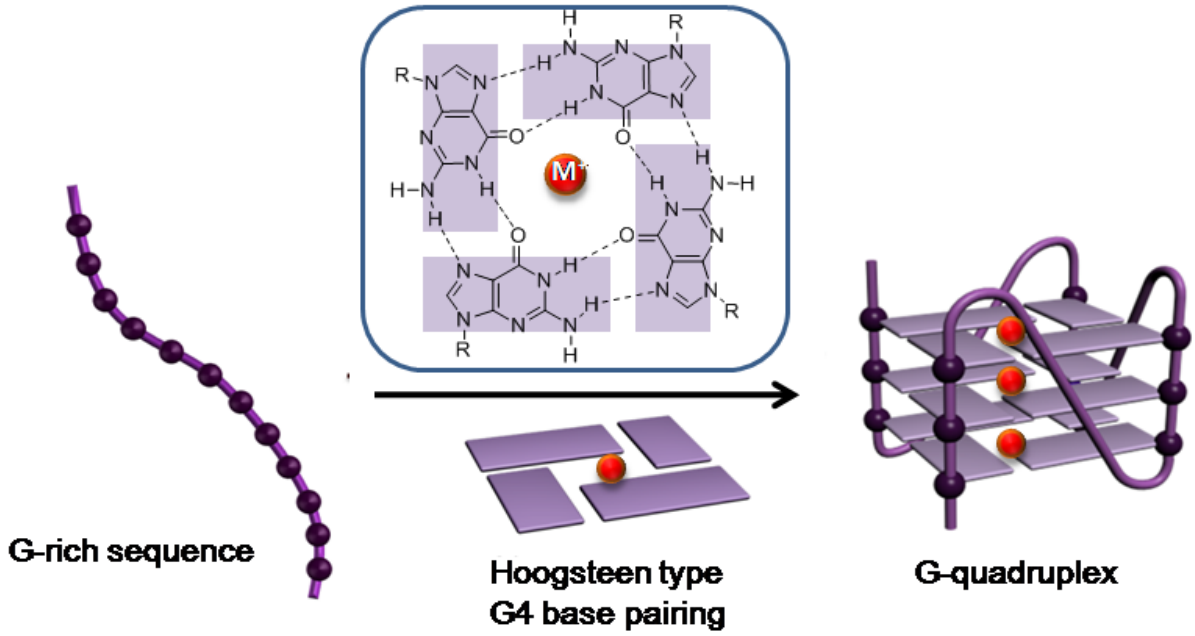

Figure 1. Structure of G-quadruplex DNA.

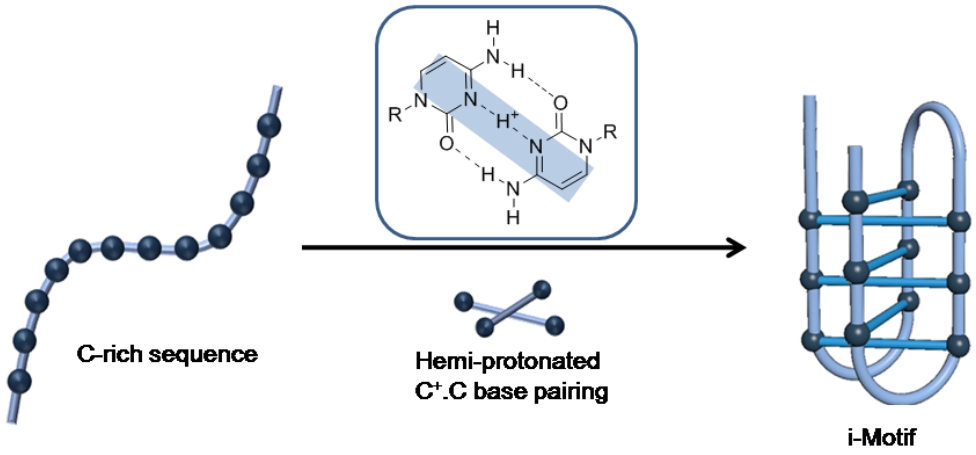

Figure 2. Structure of i-motif DNA.

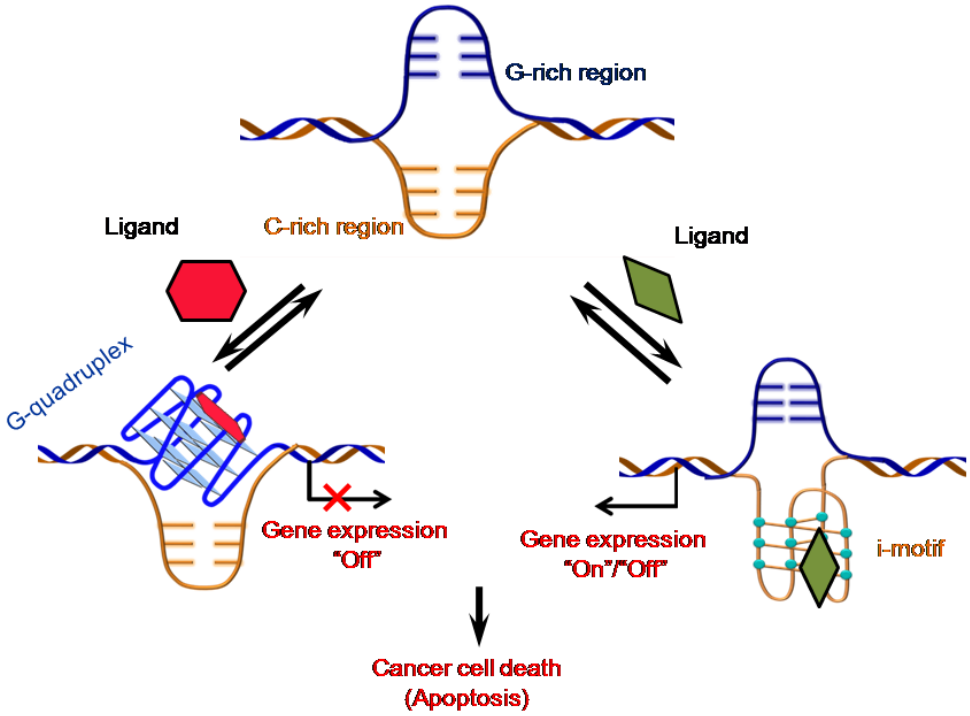

Figure 3. Regulation of gene expression by G4 and i-motif.

sometimes more stable than their DNA counterparts.[20] In comparison, cytosine-rich DNA sequences adopt i-motif structures at mildly acidic pH. An i-motif consists of two parallel-stranded duplexes intercalated in an antiparallel orientation and held together by hemi-protonated cytosine-cytosine$^+$ (C:C$^+$) basepairs[3,21] (Figure 2). Gehring *et al.*[3] first discovered these unconventional i-motif secondary structures. Studies on i-motif were previously limited based on the assumption that they are stabilized under slightly acidic conditions and are not physiologically relevant. Recent studies indicate that these non-canonical DNA structures can be formed at neutral pH[22], under conditions of negative superhelicity[23], and molecular crowding conditions[24]. Very recently, the occurrence of functional i-motifs in human cell nuclei has been proven by using a specific antibody (iMab).[4] In principle, the complementary strand of any G-quadruplex forming sequence is susceptible to form i-motifs. Thus, like G-quadruplex structures, these cytosine stretches are enriched in the promoter regions of several oncogenes (*BCL-2, c-MYC, c-KIT, KRAS, PDGF, c-MYB, HIF-1α*) within the genome and in the telomeres, i.e., the

terminal regions of chromosomes, indicating its potential role in gene regulation.[25-28] The cytosine-rich repeats in RNA can also form i-motif, but they are highly unstable.[29]

It is now well-known that G-quadruplex and i-motif structures may play complementary roles in regulating gene expression (Figure 3). G-quadruplexes are mostly believed as a repressor of gene expression, whereas stabilization of i-motifs is mainly associated with transcriptional activation.[30] The contradictory biological role of i-motifs and G-quadruplexes is coordinated in living cells by the simultaneous or mutually exclusive formation of these structures.[30] Therefore, targeting G-quadruplex or i-motif with small molecules may lead to a prospective cure for cancer and other genetic diseases. Hence, these non-canonical DNAs are potential targets for drug design and modulation of gene expression.

Targeting G-quadruplexes and i-motifs with small molecules

In comparison to widely known G-quadruplex binding molecules[31-33], very few i-motif binding compounds are reported in the literature[8]. With the genome primarily existing in a duplex structure, it is of utmost importance that a ligand should specifically bind towards a particular G-quadruplex or i-motif structures and show little or no interactions with double-helical DNA. Additionally, the ligands should have amenable properties, for instance, stability under physiological conditions, low toxicity, and specific interaction with a particular quadruplex/i-motif topology in the biological system. This would provide a reasonable basis for a therapeutic window, where the molecules interact with the target DNA secondary structures without too many undesirable side effects.

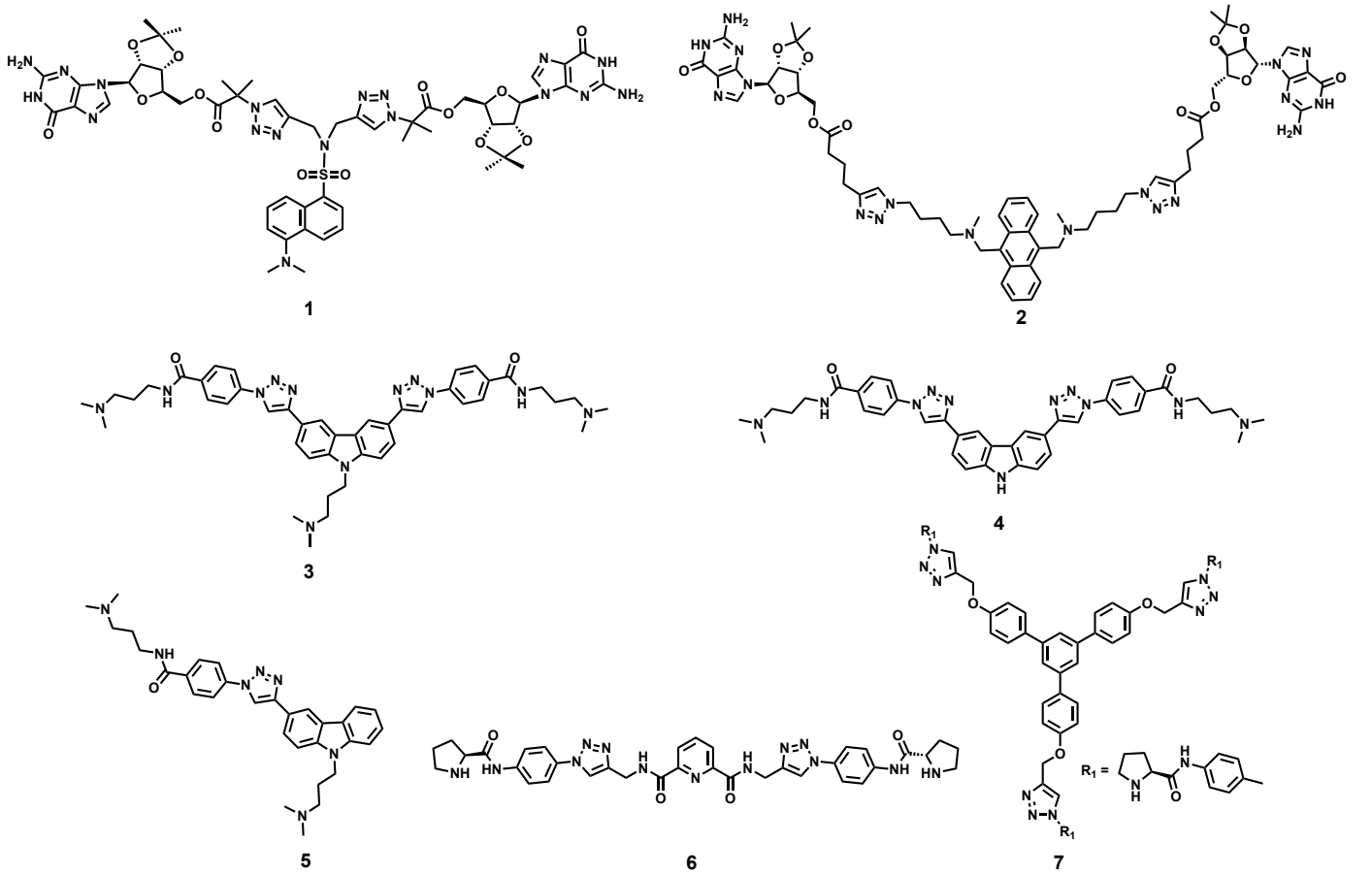

Figure 4. Structure of ligands (**1**-**7**).

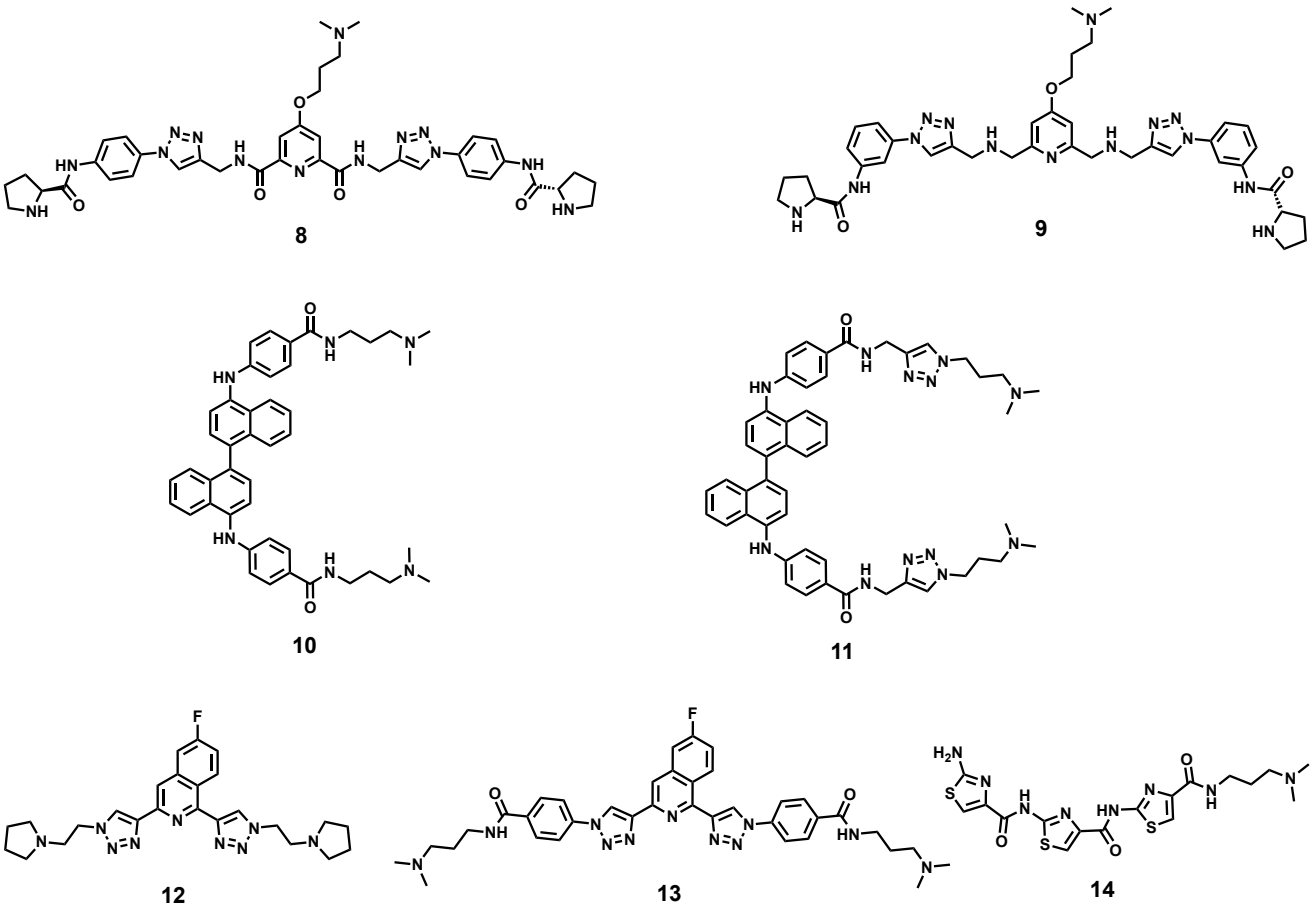

Figure 5. Structure of ligands (**8-14**).

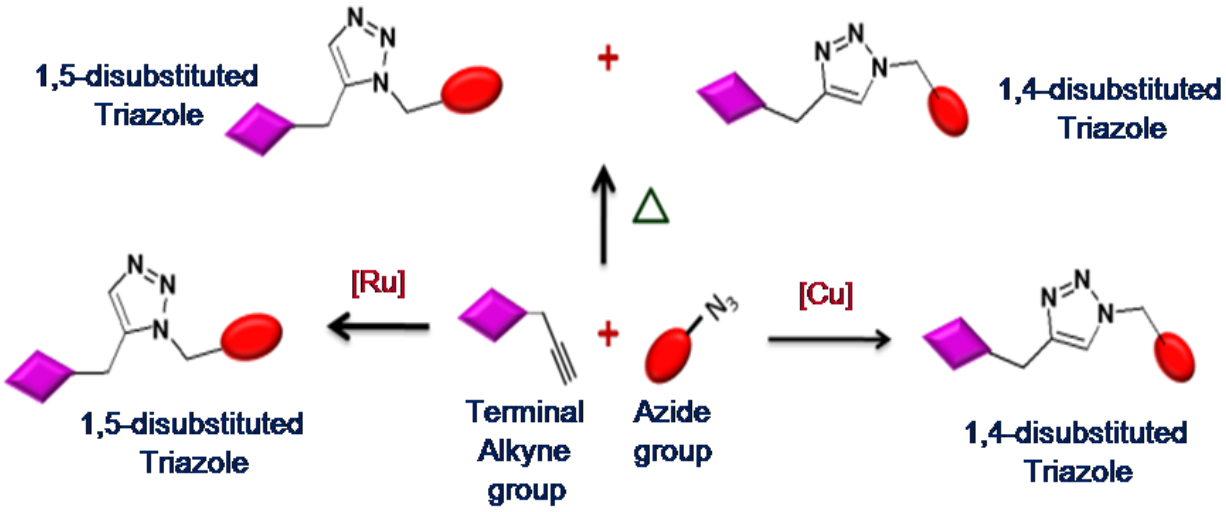

Figure 6. Azide-alkyne cycloaddition.

Rational design of ligands

We herein focus on ligands developed by our group (Figures 4 and 5). We have used copper (I) catalyzed Huisgen1,3-dipolar cycloaddition (CuAAC) to synthesize a series of compounds as selective G-quadruplex DNA binders. CuAAC is the most commonly used click chemistry transformation, a ring-forming reaction between an azide and a terminal alkyne that exclusively generates a 1,4-disubstituted 1,2,3-triazole at room temperature in the presence of copper (I) salts[34] (Figure 6). This reaction was discovered independently by Sharpless[34] and Meldal[35] in 2002. CuAAC reaction has several advantages such as operational simplicity, specificity, orthogonality, modularity, and biocompatibility.[36] Moreover, this reaction can be performed in aqueous media under mild conditions, and the azide and alkyne building blocks are either commercially available or easily synthesizable. We have employed click chemistry to functionalize different heteroaromatic moieties with appropriate side chains to generate triazole ring systems that display efficient binding abilities for DNA quadruplexes of different topologies and show significant biological activities in cellular systems.

Nucleoside conjugated triazole ligands

Using Cu (I)-catalyzed 1,3-dipolar azide-alkyne cycloaddition, Dash and coworkers designed flexible ligands by linking fluorescent dansyl[37,38] or anthracene[39] probe between two guanosine units. Both dansyl DDG (**1**) and anthracene ADG **(2)** diguanosine derivatives exhibit high binding affinity for the *c-MYC* G-quadruplex and inhibit cellular proliferation in cancer cell lines. Different biological analyses like dual luciferase assay and qRT-PCR established that these ligands repress *c-MYC* gene expression by promoter G-quadruplex stabilization. It has further been found that ADG **(2)** exhibits a profound effect on *c-MYC* related cellular events as it suppresses the transcription and translation of *hTERT* and *BCL2* genes in a quadruplex-independent manner leading to the inhibition of telomere elongation and activation of apoptotic cascades in cancer cells. ADG **(2)** thus acts as a smart *c-MYC* G4 ligand that represses the *c-MYC* expression, modulates *c-MYC* related cellular pathways, and inhibits cancer cell proliferation.

Carbazole derived triazole ligands

Dash and coworkers have developed several carbazole-derived triazole ligands by employing Cu (I) catalyzed azide and alkyne cycloaddition for targeting G-quadruplexes. Carbazole is a pharmacologically active molecular scaffold[40] with a planar heteroaromatic core, which is considered as an attractive template for designing G4 ligands[41].

Bis-triazolylcarbazoles have been synthesized by employing Cu (I) catalyzed azide and alkyne cycloaddition for targeting G-quadruplexes. One representative bis-triazolylcarbazole derivative, BTCf **(3)**, exhibits microenvironment-sensitive fluorescent properties and stains the nucleus in living cells.[42] The ligand shows a highly selective "turn-on" fluorescence response for the *c-MYC* quadruplex over duplex DNA in the presence and absence of K^+ ions. BTCf **(3)** also downregulates the *c-MYC* expression at the mRNA level and at the protein level as evidenced by the qRT-PCR and Western Blot analysis, respectively, promoting cancer cell death by apoptosis in liver cancer cell lines.[42]

Dash and coworkers reported a carbazole derivative BTC **(4)** that can induce changes in the structure and dynamics of G-rich DNA sequences in the *c-MYC* promoter region.[43] Biophysical methods like single-molecule Förster resonance energy transfer (sm-FRET), fluorescence correlation spectroscopy (FCS) as well as ^{1}HNMR studies indicated that BTC **(4)** could induce unfolded ensembles of *c-MYC* and *h-TELO* sequences into stable folded conformations in the absence of K^+ ions.[43]

Later, the group developed monotriazolylcarbazole derivatives by Cu (I) catalyzed cycloaddition.[44] In the ligand series, Cz1 **(5)** strongly interacts with *c-MYC* quadruplex DNA and inhibits its expression in the cellular system. The induction and stabilization of *c-MYC* G4 by Cz-1 **(6)** in the cellular system have also been established by monitoring the colocalization of Cz-1**(6)** with the quadruplex binding antibody BG4.[44]

Amino-acid functionalized triazole ligands

Dash *et al.* also developed prolinamide derived peptidomimetics using 'Click Chemistry' between azido prolinamides with aromatic/heteroaromatic di and tri-alkynes.[45] Different biophysical techniques indicated that ligand **6** shows excellent selectivity for *c-KIT1* quadruplex over duplex DNA and other G-quadruplexes like *c-MYC* and *h-TELO*. Compound **6** also exhibited significant antiproliferative activities against liver cancer cells by inducing necrotic cell death.[45]

Ligand Pro-4 **(7)** significantly binds and stabilizes *c-MYC* G4 over duplex DNA. *In vitro* cellular assays revealed that triazolyl tris-prolinamide Pro-4 **(7)** is cytotoxic towards liver cancer cells and is able to suppress the *c-MYC* expression at both transcriptional and translational levels.[46]

Peptidomimetic ligands, PBP1 **(8)** and PBP2 **(9)**, exhibit distinguishable recognition between i-motifs and G-quadruplexes in the promoter region of the *BCl2* gene.[47] Interestingly, these ligands can induce G-quadruplex or i-motif structures from the unstructured single-stranded DNA conformations in the absence of metal ions. PBP1**(8)** shows high selectivity for i-motifs and upregulates *BCL2* gene expression by targeting *BCL2* promoter i-motifs, exhibiting significant antiproliferative activities in different cancer cells.[47]

In 2020, Dash *et al.* reported that the prolinamide-derived peptidomimetic ligand PBP2 **(9)** binds selectively to G-quadruplex structures and could induce synthetic lethality in MCF7 breast cancer cells by repressing both *c-MYC* and *BCL2* gene expressions.[48] A few G4 ligands are known to display synthetic lethality[49,] and this phenomenon is needed to be explored for developing anti-cancer drugs.

Binaphthylamine ligands

In 2016, Dash and coworkers designed two novel fluorescent binaphthyl-amines **10** and **11** for targeting *c-MYC* G-quadruplex structure.[50] Ligand **10** containing triazolyl side chains shows a ~5 fold higher affinity for the *c-MYC* G4 DNA over ligand **11**, enlightening the importance of triazole motifs for quadruplex interactions. *In vitro* cellular assays in human cervical cancer cells (HeLa) and human alveolar basal epithelial cancer cells (A549) revealed that these ligands exhibit significant inhibitory effects on cancer cell growth by downregulating the *c-MYC* expression. Notably, binaphthylamines show a fluorescence "turn-on" response with *c-MYC* and are able to stain the nucleus in cells, suggesting their utility as fluorescent probes for cell imaging.[50]

6-Fluoro-isoquinolinetriazolederivatives

Two isoquinoline-based compounds IQ1 **(12)** and IQ2 **(13)** for the selective recognition of human telomeric G-quadruplex DNA by Cu (I) catalyzed azide-alkyne cycloaddition have been reported.[51] The ligand IQ1 **(12)** preferentially localizes in the nuclear regions of cells and induces apoptosis in HeLa cancer cells by inhibiting telomerase activity through selective interaction with telomeric DNA G-quadruplex.[51]

Thiazole polyamides

In 2018, a crescent-shaped thiazole peptide TH3 **(14)** has been developed that exhibits site-specific recognition of *c-MYC* quadruplex over duplex DNA.[52] The peptidomimetic is structurally related to the natural product distamycin A, a well-known G4 binder.[53] Biophysical assays revealed that the ligand **14** has a binding preference towards both mutated (*c-MYC 14/23*) and wild type *c-MYC* G-quadruplex over other quadruplexes (*c-KIT1*, *c-KIT2*, and *BCL2*). The molecule is able to penetrate the nucleus of cancer cells and exhibit potent antiproliferative activity in different cancer cell lines such as HeLa and A549 while showing negligible cytotoxicity for normal cells.

Dynamic template-assisted synthesis of selective ligands

Dash and coworkers also used target-guided combinatorial methods like kinetically controlled in-situ cycloaddition and thermodynamically controlled dynamic combinatorial chemistry (DCC) using DNA-linked gold-coated magnetic nanoparticles for synthesizing selective ligands specific for a particular G-quadruplex or i-motif structure (Figure 7).

We introduced an innovative approach for the target guided synthesis (TGS) of G-quadruplex ligands in which a Cu-free *in situ* click reaction, using DNA as a nano-template, has been employed[54] (Figure 8). The DNA nano template has been devised by immobilizing *c-MYC* G-quadruplex gold-coated magnetic nanoparticles. The DNA nano template facilitates the cycloaddition of azide and carbazole-alkyne fragments, generating selective high-affinity quadruplex ligands. The generated ligands can easily be isolated by magnetic decantation, and the G-quadruplex nano-template can be easily recovered and recycled. The primary lead compound Tz1**(15)** shows greater binding affinity for *c-MYC* G-quadruplex DNA and exhibits promising antiproliferative activity in HCT116 colorectal adenocarcinoma cancer cell line by inducing apoptosis. Using this methodology, we have also generated carbazole ligand **(16)** specific for *BCl2* G-quadruplex DNA that represses *BCL2* gene expression in the cellular system.[55]

This approach has also been used to develop specific ligands for i-motif structures.[55] The design and synthesis of ligands capable of binding to i-motifs are challenging due to the pH-dependent structural complexity of i-motif DNAs. In this regard, target guided synthesis (TGS) appears to be a promising methodology for the discovery of specific and high-affinity i-motif ligands. Dash *et al.* have used *c-MYC* and *BCL2* i-motifs as the templates to generate selective ligands from a pool of reactive azide–alkyne building blocks. Thiolated DNA targets are immobilized on gold-coated iron nanoparticles' surface to enable efficient isolation of the newly generated ligands from the solution mixture by simple magnetic decantation. The *in situ* cycloaddition provided triazole leads **(17** and **18)** for *c-MYC* and *BCL2* i-motif DNA. *In vitro,* cellular studies revealed that the *c-MYC* i-motif lead **17** downregulates the *c-MYC* gene expression whereas the *BCL2* i-motif lead **18** upregulates the *BCL2* gene expression.[55]

Apart from generating selective ligands for non-canonical DNA structures like G-quadruplex and i-motif by TGS approach, this methodology has also been used to design ligands specific for TAR RNA.[56] Human immunodeficiency virus type-1 (HIV-1) contains a cis-acting regulatory element called TAR RNA that can form a stable hairpin structure. This highly conserved element binds to the trans-activator protein Tat and facilitates viral replication in its latent state.[57] The inhibition of Tat–TAR interactions by selectively targeting TAR RNA can, therefore, be used as an anti-HIV therapeutic strategy. Biotin-tagged TAR RNA has been used to assemble its ligands from a pool of reactive azide and alkyne building blocks. The hit triazole-linked thiazole peptidomimetic products have been isolated from the biotin-tagged target templates using streptavidin beads. The major triazole **19** generated by the TAR RNA presumably binds to the hairpin structure, showing specificity for TAR RNA over TAR DNA and effectively inhibits Tat–TAR RNA interactions.

In another study, a nano template-guided Dynamic Combinatorial Chemical method has been employed to generate specific carbazole-derived ligands for the *c-MYC* promoter G-quadruplex.[58] The gold-coated magnetic nanoparticle-conjugated G-quadruplex DNA has been used as the template for the dynamic selection of ligands from a pool of carbazole aldehyde and amine building blocks. The lead compound **20** selectively binds to *c-MYC* G4 DNA over dsDNA, suppressing the c-MYC gene expression. Moreover, the ligand **20** can enter the nucleus and induce DNA damage in cancer cells.

A pull-down screening assay using G-quadruplex DNA nanoparticles

A simple, high-throughput and reliable screening method has been developed to identify selective ligands for a particular G-quadruplex topology from a series of small molecules[59] (Figure 9). In this method, G-quadruplex linked magnetic gold nanoparticles (NP) have been used, which could efficiently select a high-affinity binder for the G4 from a pool of ligands. Unbound ligands were eliminated by simple magnetic decantation. These DNA-linked nanoparticles are easily synthesizable and can be reused, making it a cheap screening method. Besides, this technique applies to any ligands independent of their solubility, UV absorbance, and intrinsic fluorescence properties, allowing quick screening of large compound libraries. Initially, the group optimized this competitive screening method with known G4 ligands and then used a new series of G-quadruplex interactive bis-triazolyl ligands **(21** and **22)** to identify the most potent binders for *c-MYC* and *BCL2* G-quadruplexes. The identified ligands show specific binding ability for distinct G4-DNAs in the cellular system and exert significant anti-cancer activities.

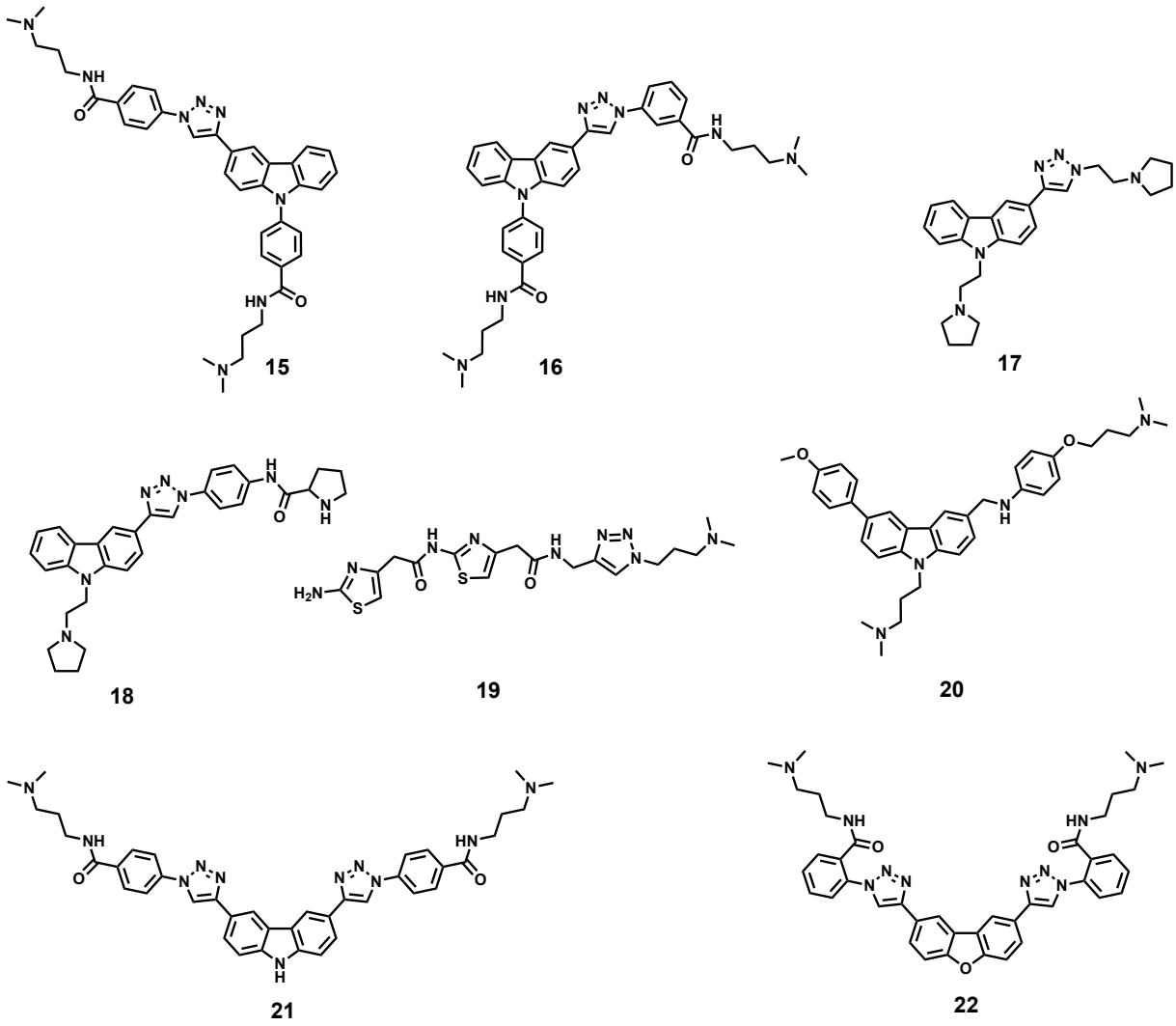

Figure 7. Structure of ligands (**15**–**22**).

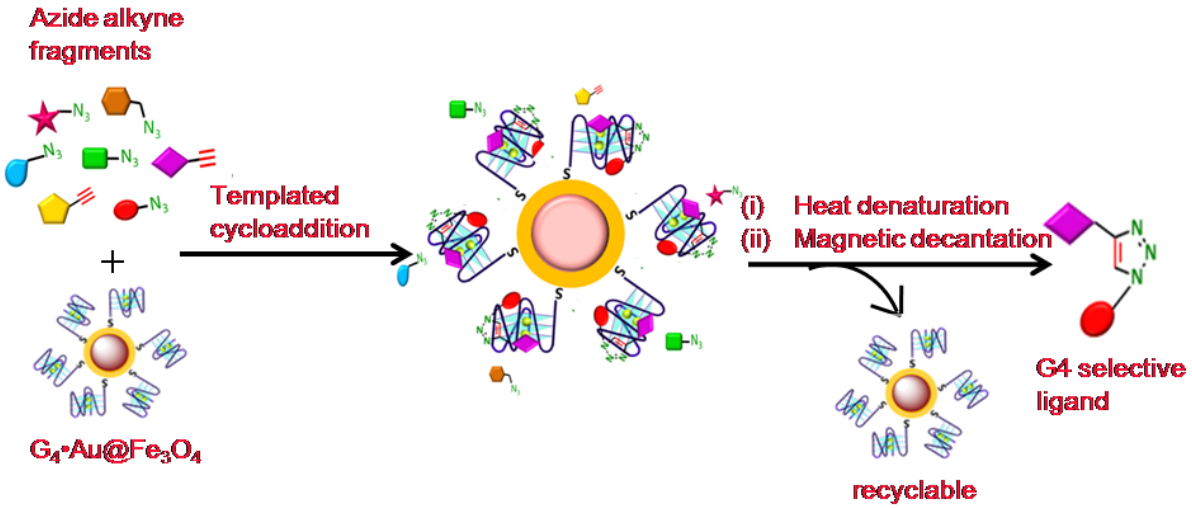

Figure 8. Target guided approach for the synthesis of selective G4 ligands.

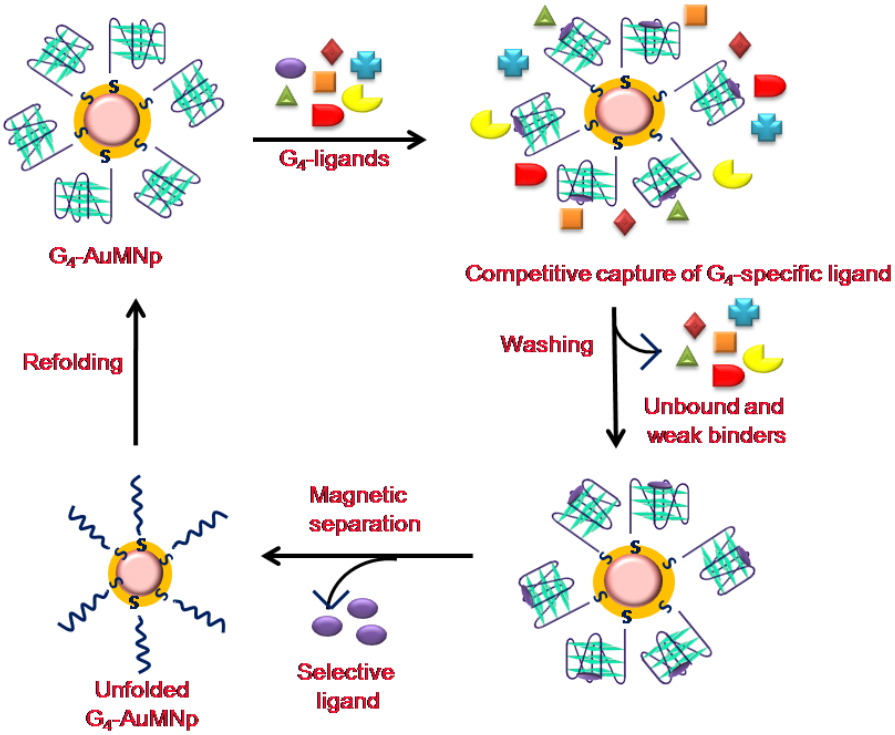

Figure 9. G4-AuMNps-mediated pull-down-based screening assay.

Bio-inspired functional architectures

DNA secondary structures are useful for biomedical applications and exhibit profound applications in the field of bio-nanotechnology. Both G-quadruplex and i-motif structures have emerged as versatile scaffolds to fabricate different programmable nanostructures. Our group has used DNA secondary structures and their components to construct bio-nanowires, logic gates, enzyme-regulated DNA-based devices, transmembrane ion channels, and hydrogels. A few DNA secondary structure interacting ligands have also been used to construct various bio-inspired devices (Figure 10).

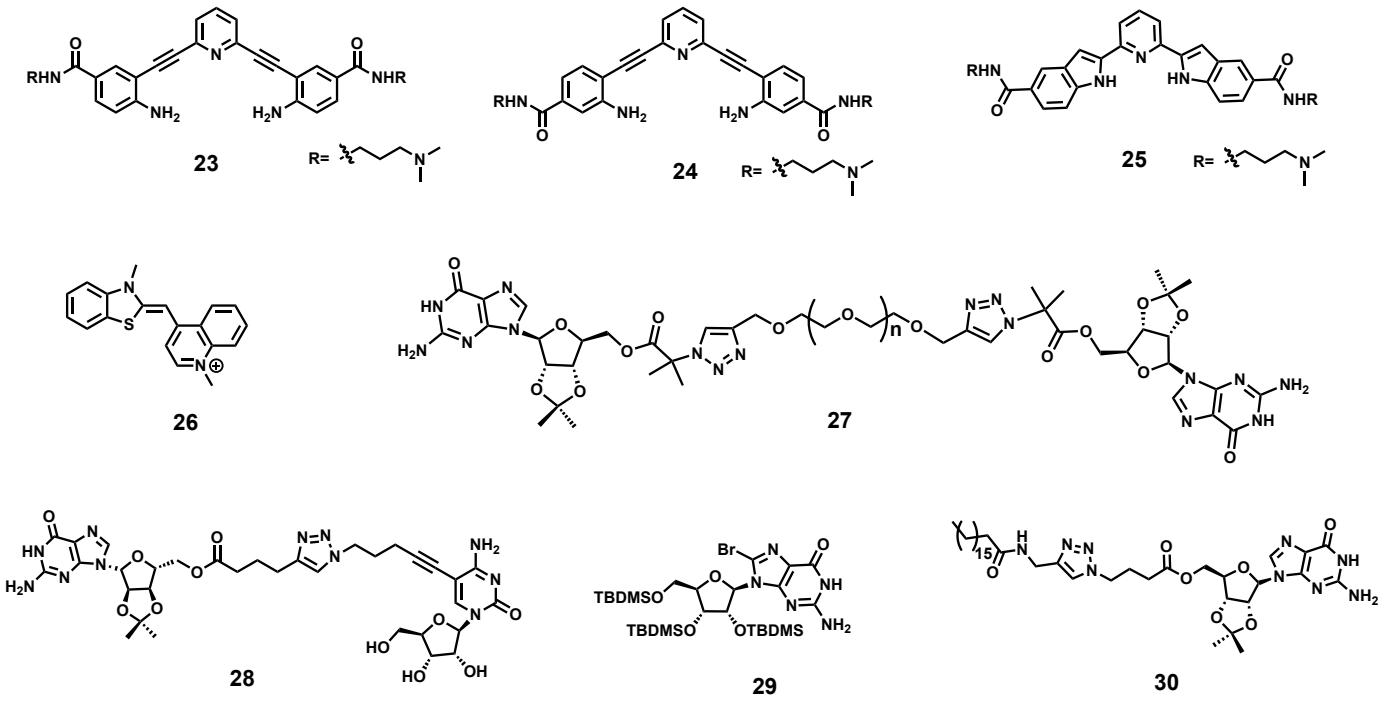

Figure 10. Structure of ligands (**23**–**30**).

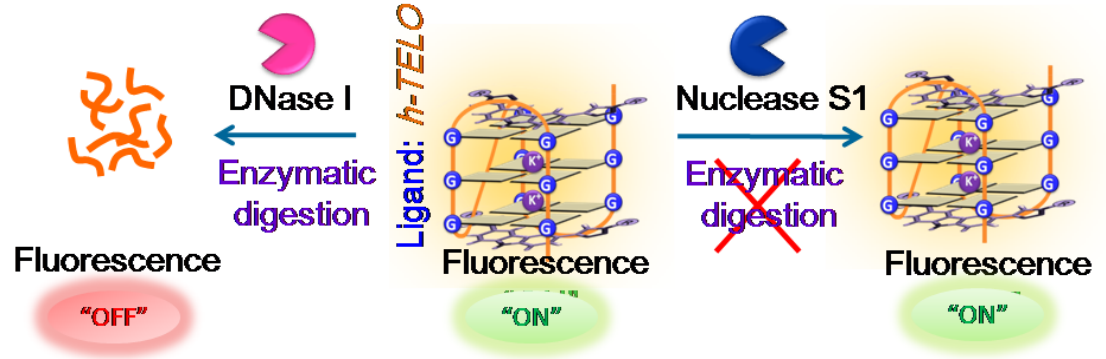

Figure 11. Differential activity of enzymes on ligand-bound DNA.

Bio-inspired nano-structures

Small molecule-quadruplex interactions have been used to construct different nanostructures like bio-nanowires, logic gates, and enzyme-regulated DNA-based devices with potential sensing and therapeutics applications.

In 2014, bis(phenylethy-nyl)pyridine carboxamides [BPEP 1 **(23)** and BPEP 2 **(24)**] have been reported to exhibit remarkable fluorescence turn-on responses upon interacting with the human telomeric G-quadruplex (*h-TELO*).[60] Different biophysical analysis demonstrated that BPEP 2 **(24)** has a high binding affinity towards h-TELO over duplex DNA. Furthermore, the ligand could induce the *h-TELO* motif by non-covalent interactions to form supramolecular polymeric nanofibers and branched nano-aggregates. This may provide a versatile platform for developing novel hybrid biomaterials with controlled structures and properties.

DNA based logic devices

G-quadruplex-small molecule interactions have also been used to fabricate DNA logic gates with pH as an external modulator.[61] Fluorescence spectroscopic study demonstrated that the fluorescence intensity of a mixture of bis-indole-based G4

ligand **(25)** and *c-KIT2* G-quadruplex DNA increases with an increase in pH and decreases with a decrease in pH. Using another 'turn-on' quadruplex binding ligand thiazole orange **(26),** a variety of logic operations (XNOR, NOR, AND, NAND, and NOT) have been devised based on the interactions of the small molecules **(25** and **26)** among themselves and with the *c-KIT2* promoter quadruplex sequence with pH as an external modulator.

In 2018, a carbazole probe **4** that exhibits distinct turn-on fluorescence responses upon interaction with *h-TELO* and nuclease enzymes (DNase I and nuclease S1) has been used to devise DNA-based logic systems.[62] Ligand **4** shows high selectivity for the mixed hybrid-type conformation of *h-TELO* G-quadruplex and can switch the antiparallel conformation of *h-TELO* to mixed hybrid type quadruplex. Ligand **4** also protects the *h-TELO* against digestion by exonucleases and nuclease S1 (Figure 11). The differential fluorescence behavior of ligand-stabilized *h-TELO* in the presence of different nucleases has been used to construct a sensor device to detect the activity of DNase I and perform various logic operations, which may help design intelligent biomolecular machines.

Ion channels

Ion channels facilitate the transport of ions across biological membranes. The development of artificial ion channels that can mimic the fundamental functions of the natural ones would be of great importance to biological research.

In 2014, Dash *et al.* synthesized bis-guanosine derivatives with covalent spacers like dansyl group, PEG, lipiphilic alkyl groups, and phenylene dicarboxamide unit by azide-alkyne cycloaddition.[63] These bisguanosine derivatives self-assemble in the lipid bilayer to form channel-like structures that could modulate the traffic of various ions (Na^+, K^+, and Cs^+) across the phospholipid bilayer. The bis-guanosine derivative **27** containing biocompatible PEG as a spacer formed large and stable pores in the membrane (Figure 12A). Notably, the conductance of the supramolecular guanosine channels in the phospholipid bilayers is inhibited by complementary cytosine. Later, in 2016, a detailed description of the synthesis and ion channel activity of artificial transmembrane ion-channels based on bis-guanosine derivatives separated by a covalent linker has been presented.[64]

The following year, an artificial transmembrane ion channel construct using a self-complementary G-C bis-nucleoside has been further developed by one-pot modular azide-alkyne cycloaddition.[65] Triazole linked guanosine-cytidine bis-nucleoside **28** can spontaneously self-assemble through H-bonding and π-π stacking to form large channels across a phospholipid bilayer and transport potassium ions (Figure 12B). It is also noteworthy that the nucleobase cytosine has inhibited the ion channel activity of this bis-nucleoside.

In 2018, the self-assembly of a lipophilic tert-butyldimethylsilyl (TBDMS) protected bromo guanosine derivative G1 **(29)** to form different nanostructures has been reported depending on incubation time.[66] G1 crystal **(29)** also exhibits strong birefringence upon exposure to polarized light, which can be used in different applications like data storage, optical devices, and bio-imaging. The supramolecular assembly of G1**(29)** can bind to aromatic dyes like rose Bengal using H-bonding and π-π stacking interactions. Further, G1 **(29)** can form discrete transmembrane ion channels in the biological membrane, enabling the transportation of potassium ions.

Very recently, the construction of an artificial ionophore using a telomeric DNA G-quadruplex and lipophilic guanosine (MG) **(30)** has been delineated.[67] Biophysical studies revealed that MG stabilizes *h-TELO* G-quadruplex by non-covalent interactions. Its lipophilic chains facilitate the insertion of the *h-TELO*G-quadruplex within the lipid bilayer to form the ionophore (Figure 12C). The ionophore preferentially transports K^+ ions across the cell membrane in different cell lines like Chinese hamster ovary (CHO) and human erythroleukemia (K-562). This study may serve as a design principle to generate selective DNA-based artificial transporters for therapeutic applications.

Hydrogel

Guanosine is known to self-assemble via non-covalent interactions like hydrogen bonding and π–π interactions and form hydrogels.[68] G-quartet, the basic building block of G-quadruplexes, can be used as a molecular template in "bottom-up self-assembly" to design hydrogels. Dash and coworkers fabricated transparent supramolecular hydrogels by potassium-ion-mediated self-assembly of guanosine and 8-bromoguanosine.[69] Remarkably, stable and functional hydrogels were formed only in the presence of both guanosine and 8-bromoguanosine, whereas the individual components precipitated within a few hours. Different bioactive dyes were found to diffuse and get released in a controlled manner through the gel, thus suggesting the potential biomedical applications of these systems. Moreover, these supramolecular structures exhibited birefringence in the presence of dyes, thus finding applications in optical devices and biomolecular imaging. In 2017, Dash *et al.* developed a novel G-quartet hydrogel construct, prepared from guanosine and phenylboronic acid in the presence of K^+ and Pb^{2+} ions[70] (Figure 13A). The K^+ stabilized hydrogel binds to iron (III)-hemin and shows DNAzyme like peroxidase activity, catalyzing the oxidation of 3,3,5,5'-tetramethylbenzidine (TMB) in the presence of H_2O_2. Furthermore, the conformation of the G-quartet assemblies in the hydrogel can be altered by varying the K^+ and Pb^{2+} ions. This conformational switching has been used to devise a molecular logic gate for sensing toxic Pb^{2+} ions. This hydrogel construct thus provides a three-in-one platform for catalysis, sensing, and logic operation. In another study, cytidine nucleoside, boronic acids have been used to prepare hydrogel in the presence of Ag^+ ions[71] (Figure 13B). These hydrogels, presumably formed by an i-motif like arrangement of cytidine and its boronate ester analogs, possess excellent thixotropic and self-healing properties. Moreover, these hydrogels show potent antibacterial activities against various Gram-negative bacteria.

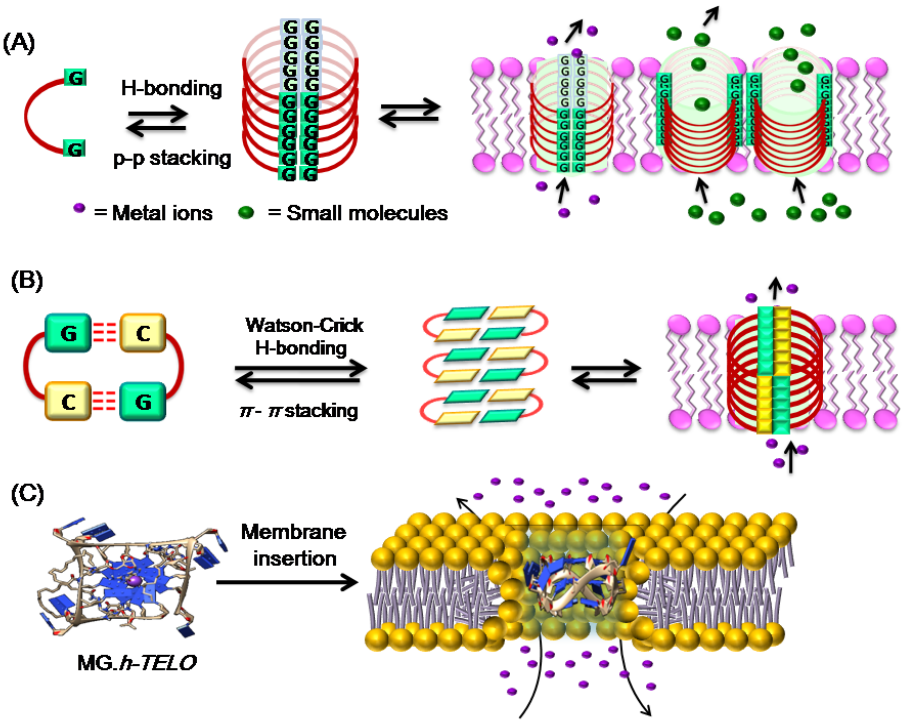

Figure 12. Artificial ion channels based on nucleoside derivatives.

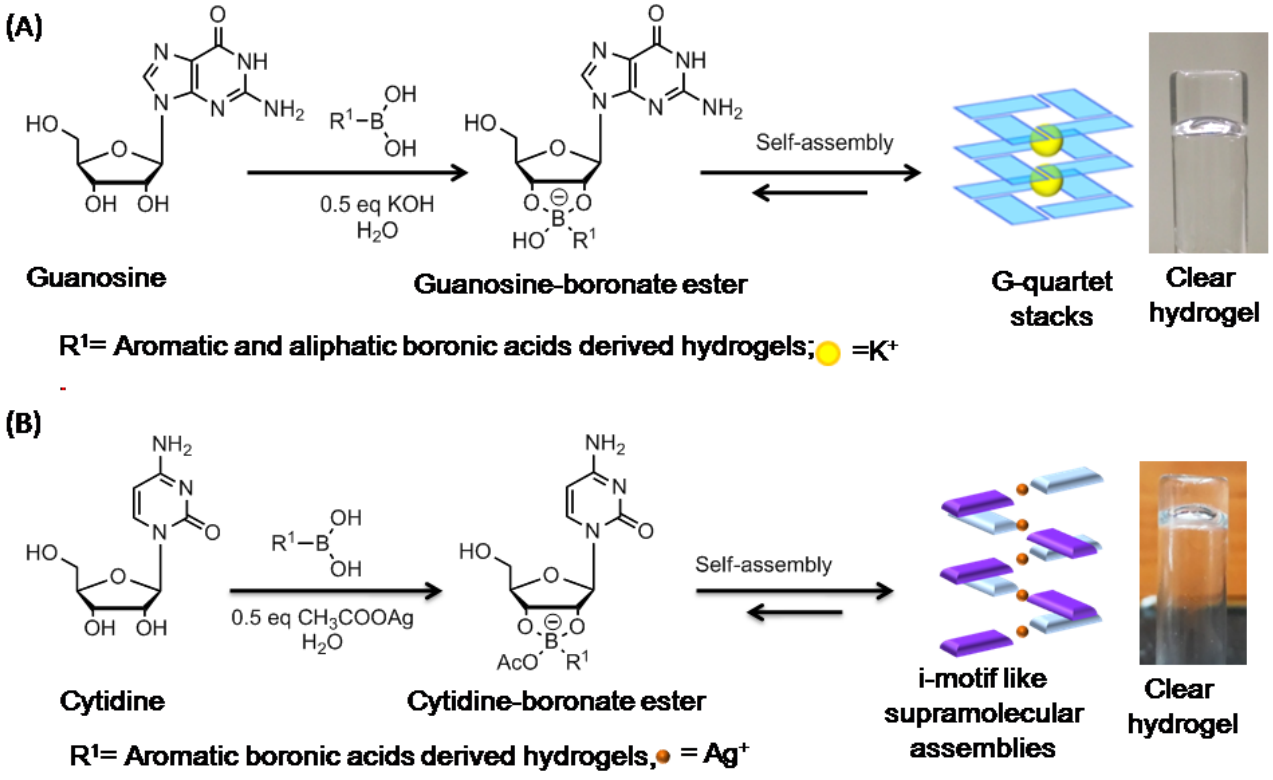

Figure 13. Nucleoside-derived supramolecular hydrogels

Conclusion and Future Perspectives

In conclusion, DNA secondary structures like G-quadruplexes and i-motifs can be chemically manipulated for therapeutic and nano-biotechnological applications. We have described the development of small-molecule ligands in our laboratory that selectively target G-quadruplex/i-motif structures over duplex DNA. These ligands modulate the gene expression by stabilizing DNA secondary structures and regulating different biological functions, which could help anti-cancer therapeutics. We have also highlighted the construction of G-quadruplex-based functional nanostructures by non-covalent interactions of DNA quadruplexes with small molecules, having potential applications in drug delivery and DNA-based logic sensors.

G-quadruplexes and i-motifs have emerged as attractive targets as well as tools in anti-cancer drug development. In the last few decades, a flurry of activities in the synthesis and development of suitable ligands that specifically target G-quadruplex/i-motif structures and modulate gene expression have been reported. Various chemical approaches have been employed for tuning the specificity of these ligands against a particular G-quadruplex or i-motif structure. Recently reported molecules can be explored for preclinical and clinical trials and may be used as potential therapeutic candidates to treat human cancers. Besides, many elegant reports have revealed the potential of these noncanonical nucleic acid structures to design nanoscale structures and devices which may found profound applications in bio-sensing, molecular computing, and therapeutic applications. Although there is much to be understood about these non-canonical DNA structures, the successful design of molecular probes and G-quadruplex-based functional nanostructures discussed above will undoubtedly lead to many new opportunities and innovative applications for developing the next generation therapeutics and nanodevices.

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