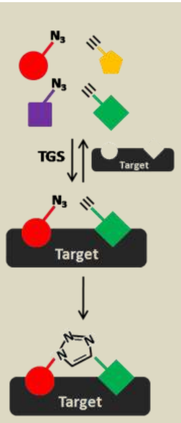
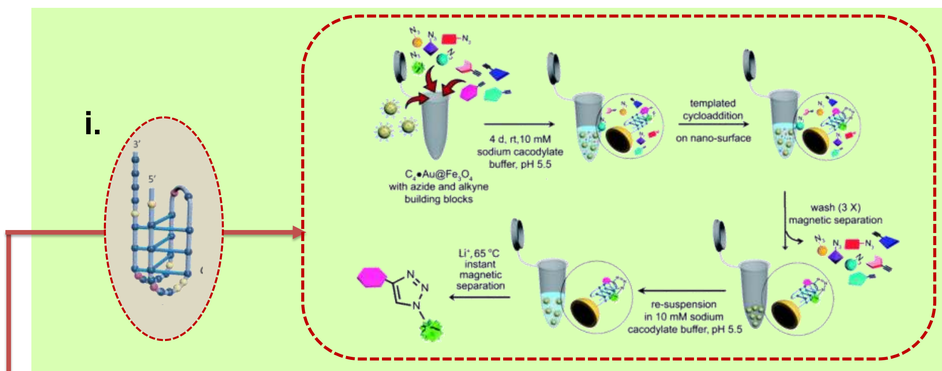
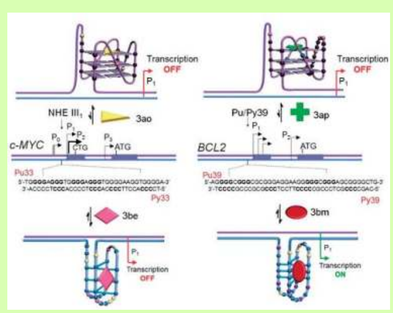
Bioorthogonal Synthesis of Molecular Probes for Modulating the Structure and Function of Non-canonical Nucleic Acids

Objectives: (1) Target-guided synthesis of selective molecular probes for targeting non-canonical nucleic-acids (NA). (2) Biological function of ligand-NA interaction on the expression of genes of clinical interest. (3) Construction of programmable biomolecular devices using small molecule-NA interactions.

Target Guided Synthesis (TGS)

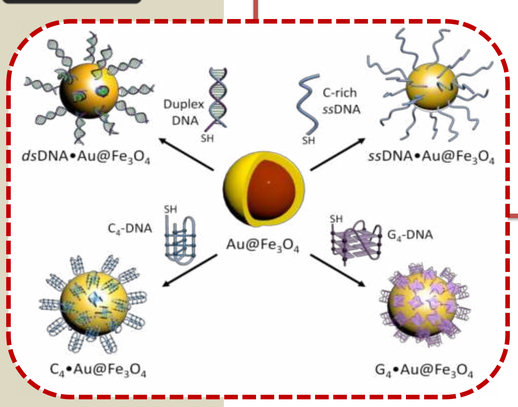


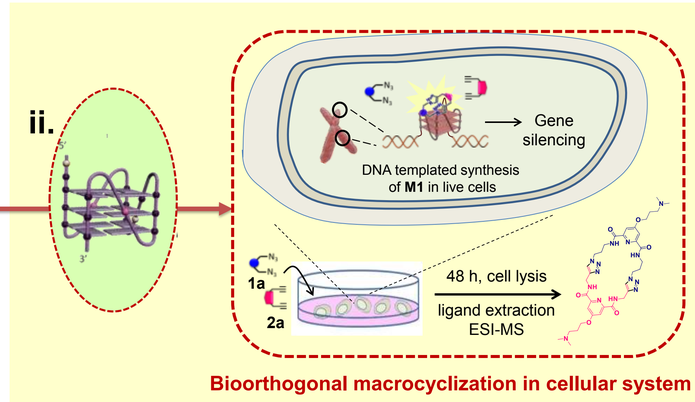


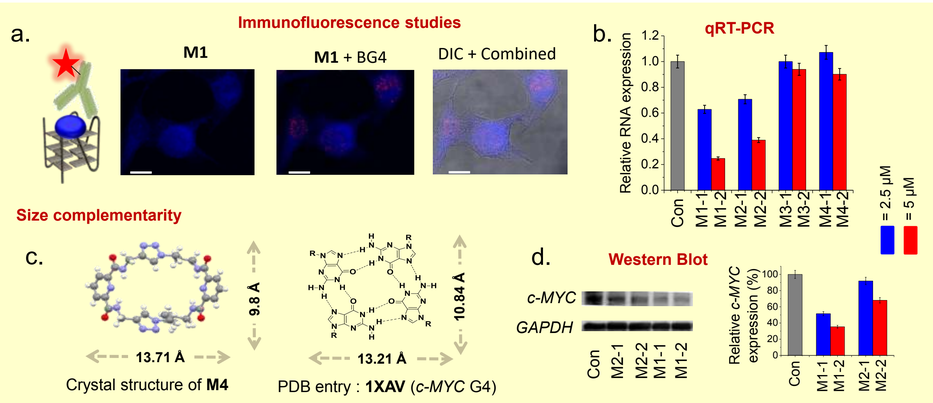


- *c-MYC* and *BCL2* i-motifs and the corresponding G4s, grafted on gold-coated magnetic nanoparticles, direct the metal-free synthesis of i-motif specific ligands.
- Lead compounds modulate the transcription of *c-MYC* and *BCL2* genes.
- Enables cost-effective and rapid synthesis of highly potent and selective drug candidates for non-canonical DNA motifs.
- Combines synthesis and screening without the need to synthesize an extensive ligand library.

Saha P, Panda D, Müller D, Maity A, Schwalbe H, Dash J. In situ formation of transcriptional modulators using non-canonical DNA i-motifs. *Chem. Sci.,* 2020,11, 2058-67







Chaudhuri R, Prasanth T, Dash J. Expanding the toolbox of target-directed bio-orthogonal synthesis: In situ direct macrocyclization by DNA templates. *Angew. Chem., Int. Ed.,* 2023, 62, e202215245

Bioorthogonal reactions:

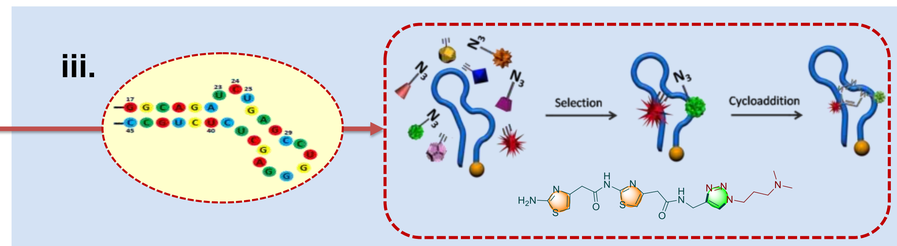
1) reaction under physiological conditions

2) inert towards biological processes

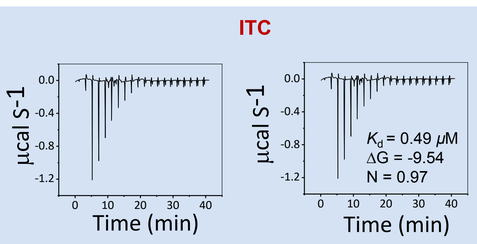
3) fast reaction kinetics at low reactant conc.

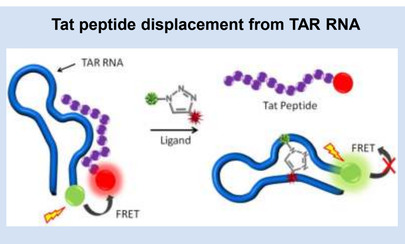
4) a single stable product

5) non-toxic



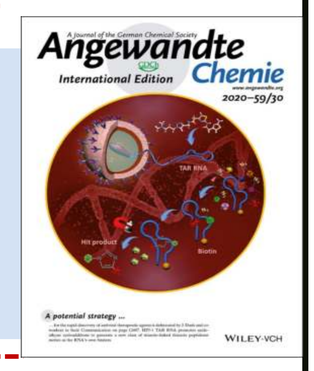
❖ Rapid access to novel thiazole based peptidomimetics as HIV-1 TAR-Tat inhibitors.



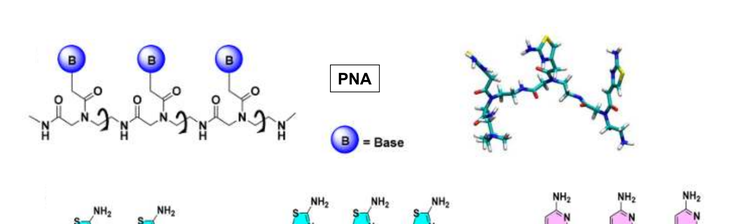


❖ Thiazole peptidomimetic strongly interacts with the bulge region of TAR RNA and effectively inhibits the Tat-TAR RNA interactions.

Paul R, Dutta D, Paul R, Dash J. Target-directed azide-alkyne cycloaddition for assembling HIV-1 TAR RNA binding ligands. *Angew. Chem., Int. Ed.* 2020, 132, 12507-11



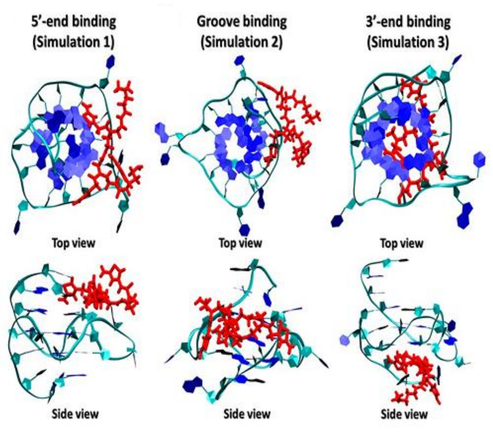
PNAs: A Promising Therapeutic Tool in Cancer Treatment



Synthesis of a thiazole modified PNA mimic

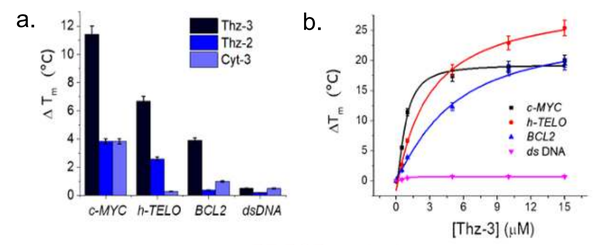
- offers flexible binding sites
- selectively recognizes and stabilizes of *c-MYC* G-quadruplex DNA with turn-on fluorescence
- cell permeable
- regulates gene expression

MD Simulation

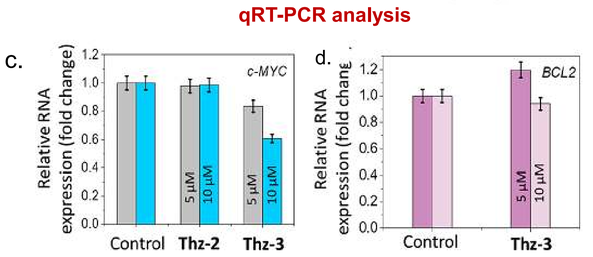


❖ MD simulations reveal that Thz-3 binds to *c-MYC* G4 DNA with a 1:3 binding stoichiometry at the 5' and 3'-terminal tetrads and the groove region, forming stable complexes by electrostatic interactions and π-π stacking.

FRET Melting assay



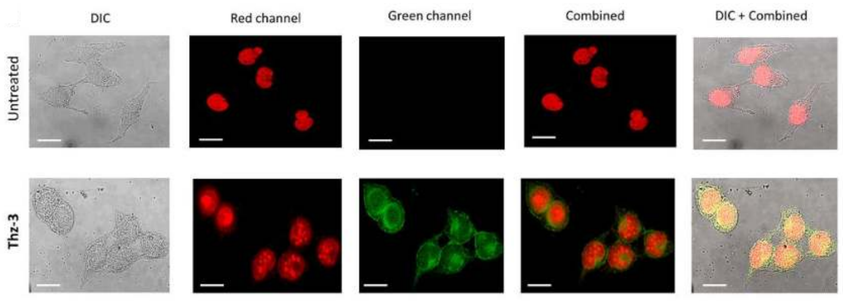
qRT-PCR analysis

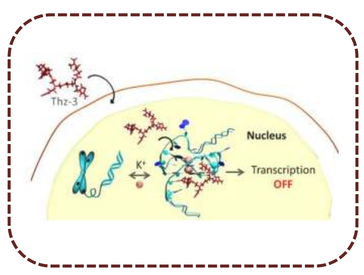


❖FRET melting analysis and fluorimetric titration studies collectively indicate Thz-3 as a potential *c-MYC* G4 DNA binder.

❖ Thz-3 regulates gene expression at the mRNA level, downregulating *c-MYC* gene expression

Confocal microscopic images



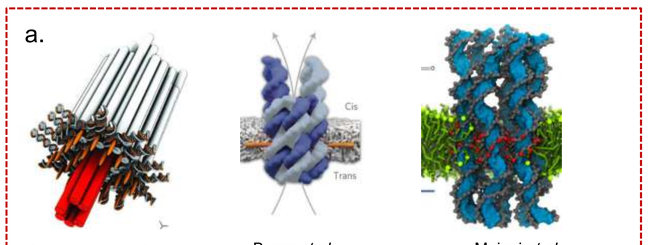


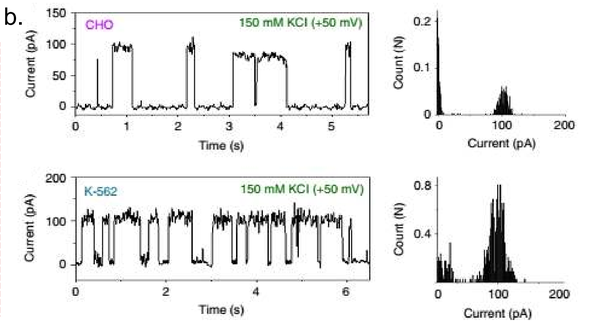
Confocal images show localization of Thz-3 within the nucleus of HeLa cells (green fluorescence)

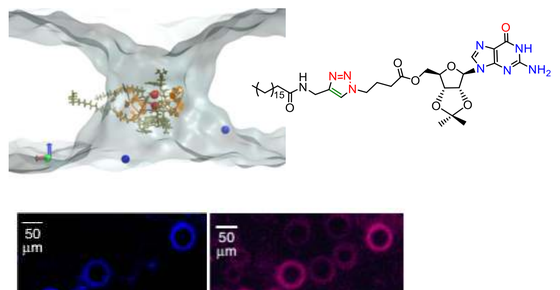
Dash J. ***Bioconjug. Chem***. 2022, *33*, 1145-55.

Construction of biomolecular nano-devices

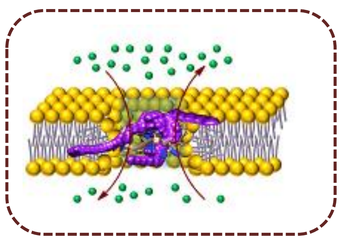
a. G4 DNA Based Cation-Specific Ion-channel



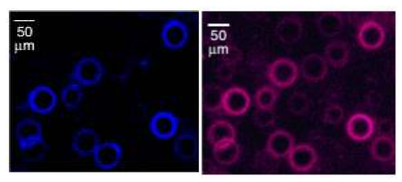




❖ Voltage-clamp experiments demonstrate that *h-TELO* ionophore transports K^+-ions across Chinese hamster ovary (CHO) and human erythroleukemia (K-562) cell membranes.



❖ Selective transport of K^+ ion (green balls) transport within a lipid membrane by *h-TELO* G-quadruplex-lipophilic guanosine ionophore (purple).

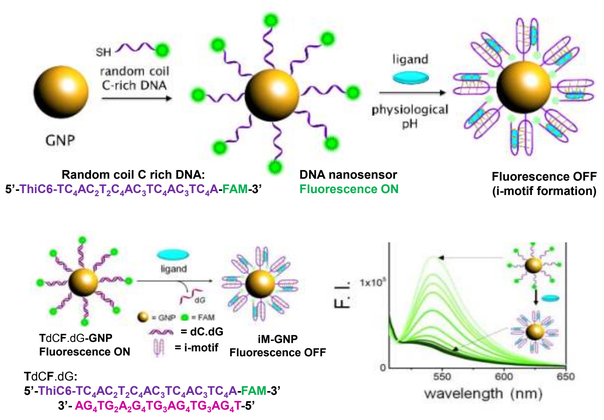


Fluorescent images of lipid vesicles (GUV) with MG/h-TELO (blue circle) and co-staining with MG/h-TELO and membrane-binding dye, Nile red (purple circle).

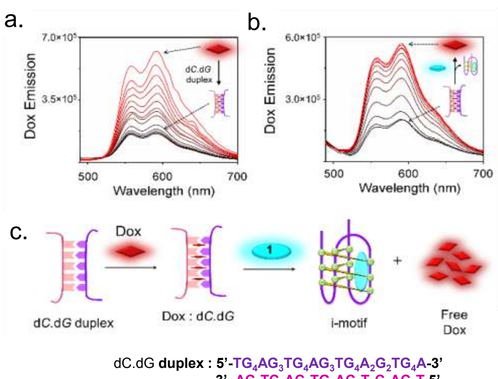
Dash J., ***Nat. Commun.*** 2020, *11*, 469.

b. A DNA nanosensor for monitoring ligand-induced i-motif formation

- a thiolated C-rich DNA grafted gold nanoparticle system as a sensor for detecting ligand-induced i-motif formation
- distance-dependent and conformation-specific fluorescence turn-off response as the readout
- screening of i-motif folding and stabilizing ligands



Fluorescence displacement of doxorubicin by i-motif ligand



dC.dG **duplex : 5'-**$TG_4AG_3TG_4AG_3TG_4A_2G_2TG_4A$**-3'**
3'- $AC_4TC_3AC_4TG_3AC_4T_2C_2AC_4T$**-5'**

- the formation of i-motif DNA from duplex DNA by a carbazole
- duplex DNA conformation is perturbed by the ligand, triggering the formation of stable i-motif DNA

Dash J.,***Org. Biomol. Chem.***, 2021, *19*, 1965-1969

Future Directions: (i) Identification and exploration of new non-canonical NA motifs in mammalian and microbial cells *(ChemRxiv, doi: 10.26434/chemrxiv-2022-sfsd4-v2),* and their cellular function; (ii) Synthesis of new probes for regulating oncogenes; (iii) *In-vivo* studies; (iv) Development of NA-small molecule nanosystems. **We thank Wellcome Trust-DBT India Alliance [Grant Number, IA/S/18/2/503986] for funding.**

Details of the research work duly signed by the applicant, for which the Sun Pharma Science Foundation Research Award is claimed, including references and illustrations (not to exceed 6000 words).

Regulation of Non-canonical Nucleic Acids Structure and Function

Nucleic acids, apart from the right-handed double-helical structure, can adopt various non-canonical secondary structures; among which G-quadruplexes and i-motifs are well-studied. 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.[1,2] They could also be involved in several cancer-related processes, thus representing attractive targets for anticancer drug discovery.[3-5]

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.[6] The stability of the G-quadruplex structure largely depends on monovalent cations, specifically K^+ and Na^+ (Figure 1). These structures are also found to be stable under physiological conditions.[7] Recent studies using G4-specific antibodies[2] and *in vivo* NMR[8] suggested their *in vivo* existence. Majority of these G4 forming sequences are prevalent within the regulatory regions of the genome, 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.[9-17] Guanine-rich RNA sequences can also form stable G-quadruplexes, which are sometimes more stable than their DNA counterparts.[18]

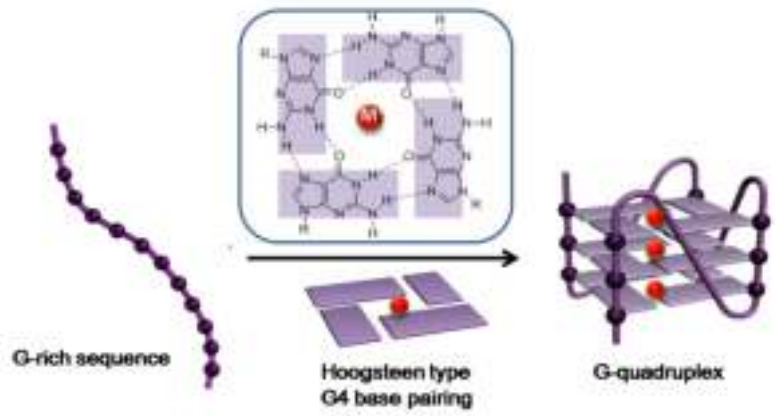


Figure 1. Structure of G-quadruplex DNA.

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[19,20] (Figure 2). Gehring*et al.*[19] 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[20], under conditions of

negative superhelicity[21], and molecular crowding conditions[22]. Very recently, the occurrence of functional i-motifs in human cell nuclei has been proven by using specific antibody (iMab).[1] 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.[23-26] The cytosine rich repeats in RNA can also form i-motif but they are highly unstable.[27]

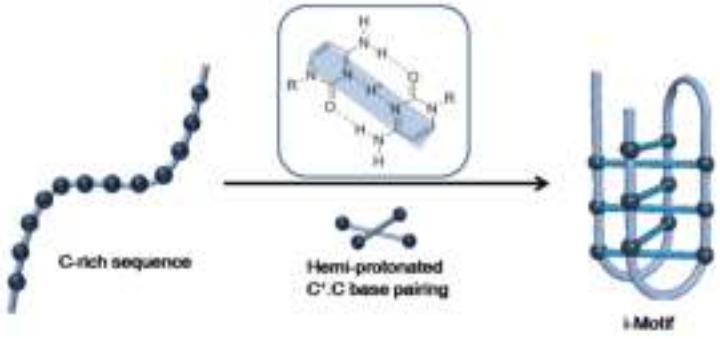


Figure 2. Structure of i-motif DNA.

It is now well-known that G-quadruplex and i-motif structures may play complementary roles in the regulation of 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.[28] 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.[28] Therefore, targeting G-quadruplex or i-motif with small molecules may lead to prospective cure for cancer and other genetic diseases and hence these non-canonical DNAs are potential targets for drug design and modulation of gene expression.

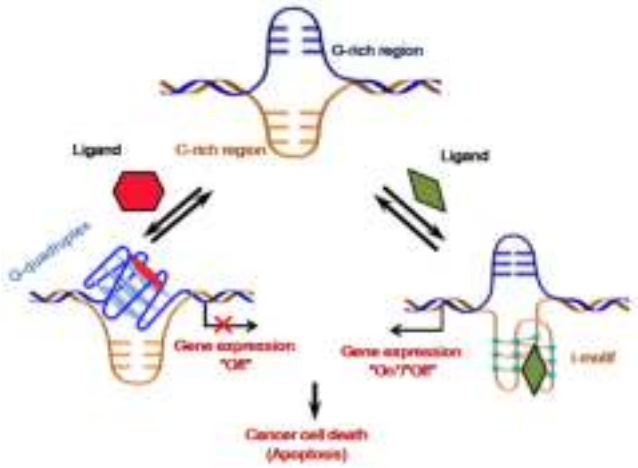


Figure 3. Regulation of gene expression by G4 and i-motif.

(A) Targeting G-quadruplexes and i-motifs with small molecules

In comparison to widely known G-quadruplex binding molecules[29-31], a very few i-motif binding compounds are reported in the literature[5]. With the genome largely 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, it is desirable for the ligands to have amenable properties, for instance, stability under physiological conditions, low toxicity and specific interaction with a particular quadruplex/i-motif topology in biological system. This would provide a good basis for a therapeutic window, where the molecules interact with the target DNA secondary structures without too many undesirable side effects.

Dr. Dash and her group used simple synthetic methods to design novel molecular probes for selectively targeting different nucleic acid secondary structures and evaluated their effects in cellular system.

(1) Rational design of ligands: Prof. Dash and her group has 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 transformations, 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[32] (Figure 6). This reaction was discovered independently by Sharpless[32] and Meldal[33] in 2002. CuAAC reaction has a number of advantages such as operational simplicity, specificity, orthogonality, modularity and biocompatibility.[34] 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. They 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.

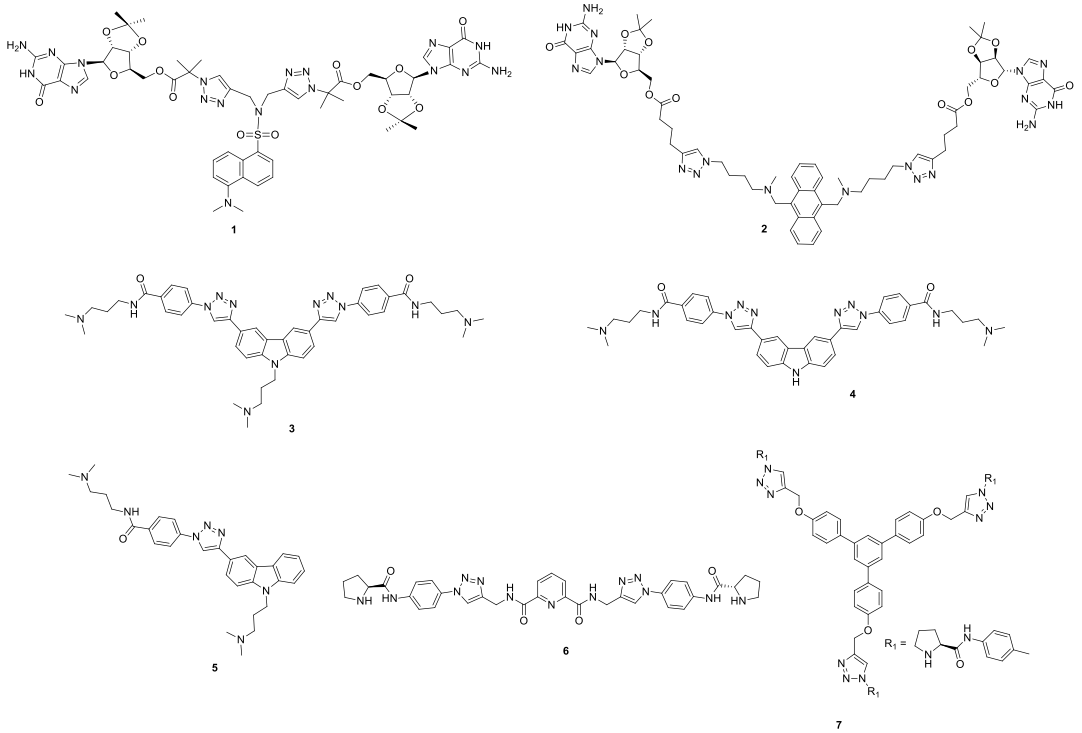


Figure 4. Structure of ligands (1-7).

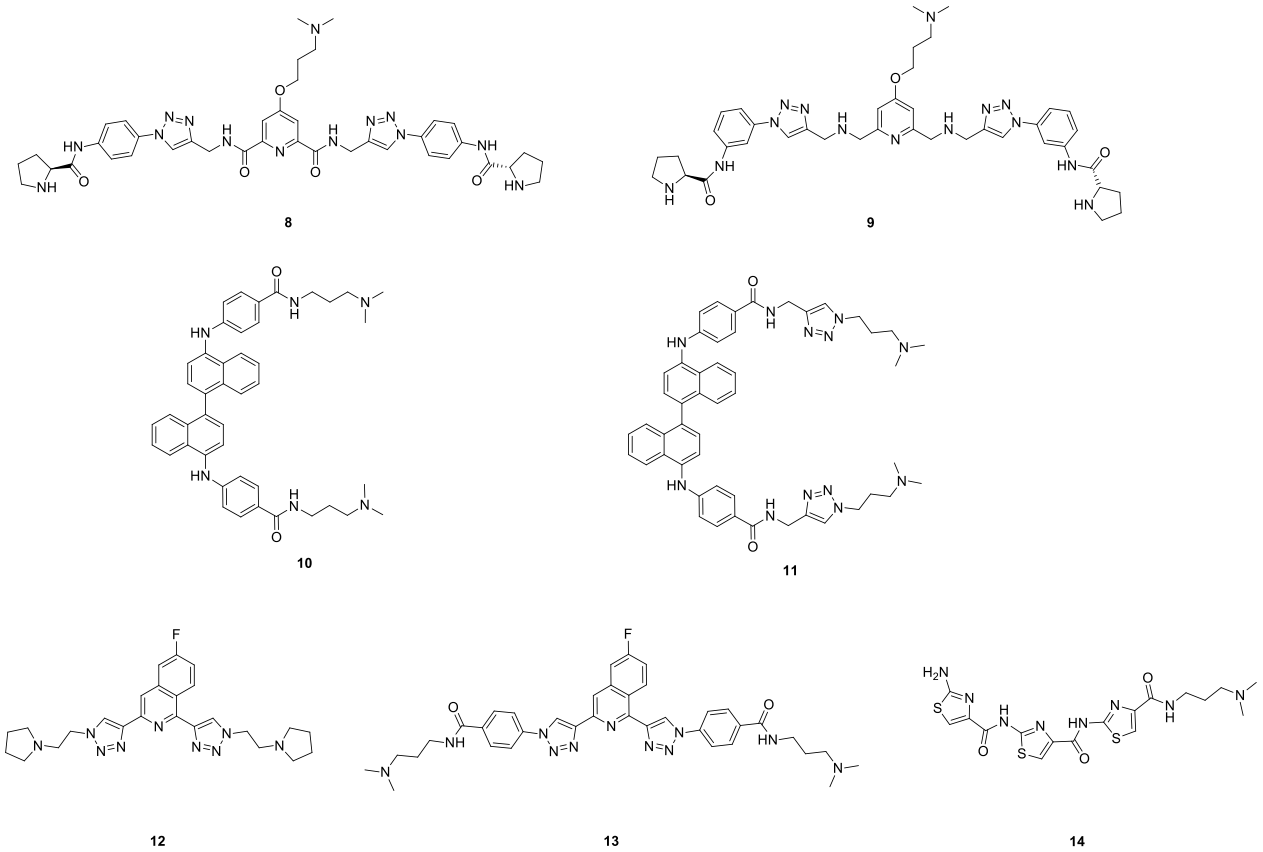


Figure 5. Structure of ligands (8-14).

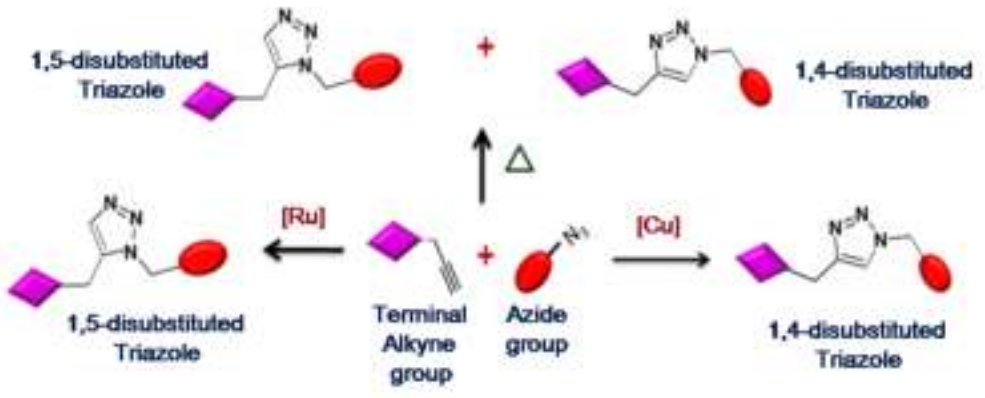


Figure 6. Azide-alkyne cycloaddition.

(a)Nucleoside conjugated triazole ligands

Using Cu (I)-catalyzed 1,3-dipolar azide–alkyne cycloaddition, Dash and co-workers designed flexible ligands by linking fluorescent dansyl[35,36] or anthracene[37] 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 analysis 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 not only represses the *c-MYC* expression but can also modulate *c-MYC* related cellular pathways and inhibit cancer cell proliferation.

(b)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[38], having a planar heteroaromatic core, which is considered as an attractive template for designing G4 ligands[39].

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 nucleus in living cells.[40] The ligand shows a highly selective "turn-on" fluorescence response for the *c-MYC* quadruplex over duplex DNA in the presence as well as in the 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.[40]

Dash and co-workers 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.[41] 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)** can induce unfolded ensembles of *c-MYC* and *h-TELO* sequences into stable folded conformations in the absence of K^+ ions.[41]

Later, the group developed monotriazolylcarbazole derivatives by Cu (I) catalyzed cycloaddition.[42] In the ligand series, Cz1 **(5)** strongly interacts with *c-MYC* quadruplex DNA and inhibits its expression in cellular system. The induction and stabilization of *c-MYC* G4 by Cz-1 **(6)** in cellular system have also been established by monitoring colocalization of Cz-1**(6)** with the quadruplex binding antibody BG4.[42]

(c)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.[43] Different biophysical techniques indicated that the ligand **6** shows excellent selectivity for *c-KIT1* quadruplex over duplex DNA and other G-quadruplexes like *c-MYC* and *h-TELO*. The compound **6** also exhibited significant antiproliferative activities against liver cancer cells by inducing necrotic cell death.[43]

Ligand Pro-4 **(7)** significantly binds and stabilizes *c-MYC* G4 over duplex DNA. *In vitro* cellular assays revealed 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.[44]

Peptidomimetic ligands, PBP1 **(8)** and PBP2 **(9)**, exhibit distinguishable recognition between i-motifs and G-quadruplexes in the promoter region of *BCl2* gene.[45] Interestingly, these ligands have the ability to 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.[45]

In 2020, Dash et al. have 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.[46] A few G4 ligands are known to display synthetic lethality[47] and this phenomenon is needed to be explored for developing anticancer drugs.

(d)Binaphthylamine ligands

In 2016, Dash and co-workers designed two novel fluorescent binaphthyl-amines **10** and **11**, for targeting *c-MYC* G-quadruplex structure.[48] 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 exhibit 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.[48]

(e)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.[49] 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.[49]

(f)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.[50] The peptidomimetic is structurally related to the natural product distamycin A, which is a well-known G4 binder.[51] Biophysical assays revealed that the ligand **14** has a binding preference towards both mutated (*c-MYC 14/23*) as well as 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.

(2) Dynamic template-assisted synthesis of selective ligands

Dash and co-workers 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).

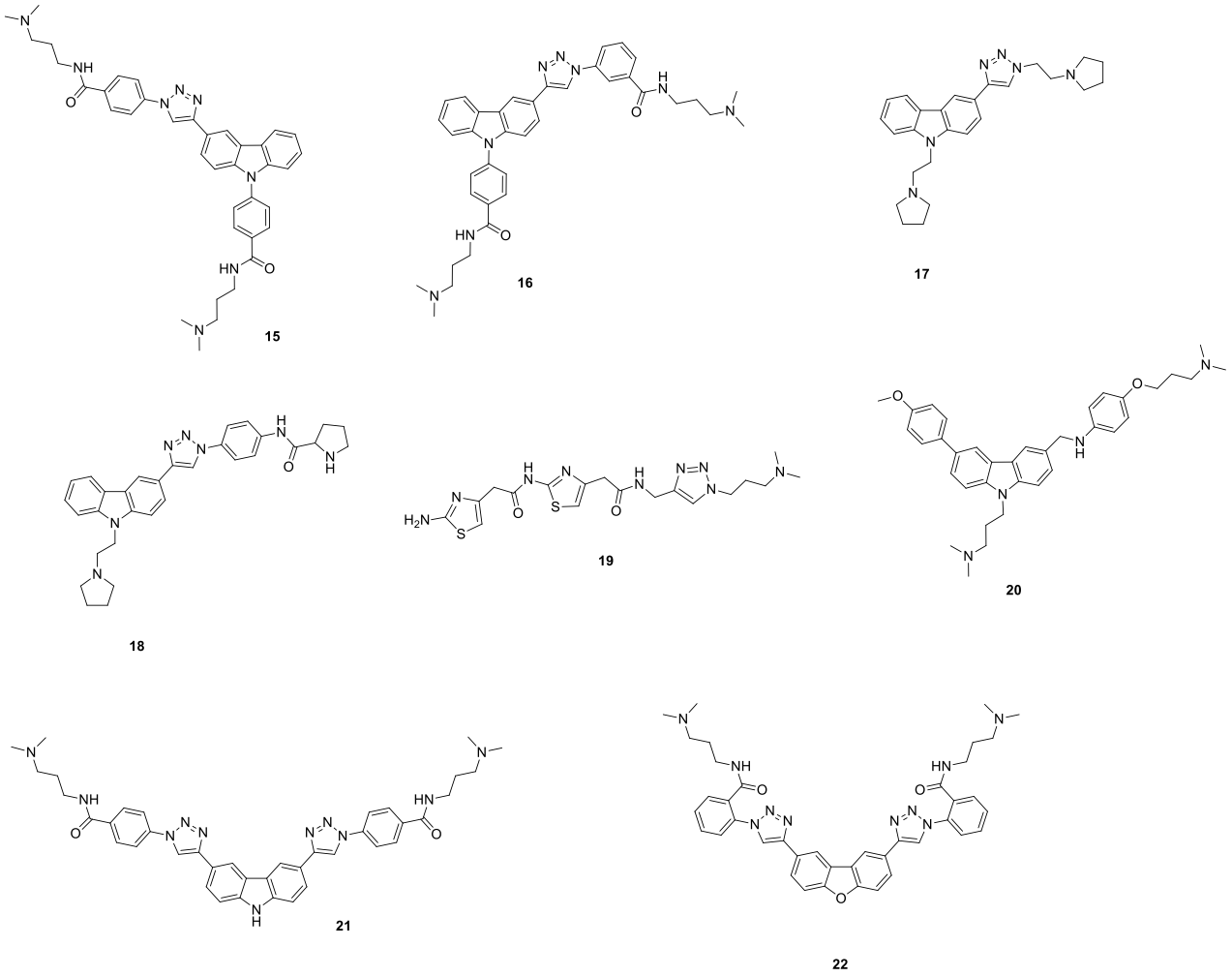


Figure 7. Structure of ligands 15– 22.

Dr. Dash and her team introduced an innovative approach for the target guided synthesis (TGS) of G-quadruplex ligands in which Cu free in-situ click reaction, using DNA as a nano-template, has been employed[52] (Figure 8). The DNA nanotemplate has been devised by immobilizing *c-MYC* G-quadruplex gold coated magnetic nanoparticles. The DNA nanortemplate 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 major lead compound Tz1**(15)** shows greater binding affinity for *c-MYC* G-quadruplex DNA and exhibits promising anti-proliferative activity in HCT116 colorectal adenocarcinoma cancer cell line by inducing apoptosis. Using this methodology, they have also generated carbazole ligand **(16)** specific for *BCl2* G-quadruplex DNA that represses *BCL2* gene expression in cellular system.[53]

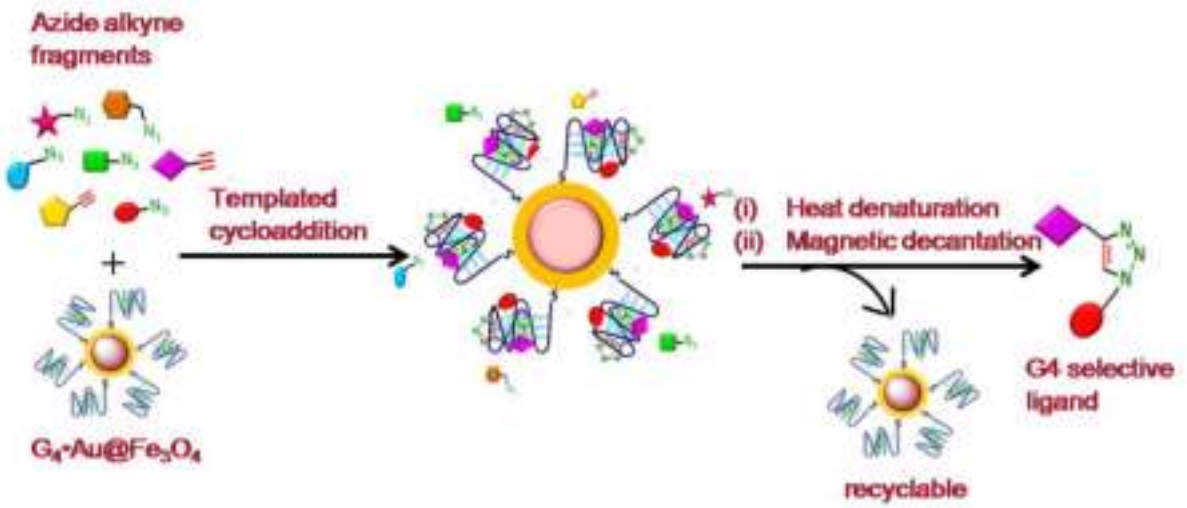


Figure 8. Target-guided approach for the synthesis of selective G4 ligands

This approach has also been used to develop specific ligands for i-motif structures.[53] 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 the surface of gold-coated iron nanoparticles 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.[53]

Apart from generating selective ligands for non-cannonical DNA structures like G-quadruplex and i-motif by TGS approach, this methodology has also been used to design ligands specific for TAR RNA.[54] 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.[55] 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 own 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 lead **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 nanotemplate-guided Dynamic Combinatorial Chemical method has been employed to generate specific carbazole derived ligands for the *c-MYC* promoter G-quadruplex.[56] 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 expression of *c-MYC* gene. Moreover, the ligand **20** can enter the nucleus and induce DNA damage in cancer cells.

(3)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[57] (Figure 9). In this method, G-quadruplex linked magnetic gold nanoparticles (NP) have been used which could efficiently select 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. In addition, this technique is applicable 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 ligands, thus identified, show specific binding ability for distinct G4-DNAs in the cellular system and exert significant anticancer activities.

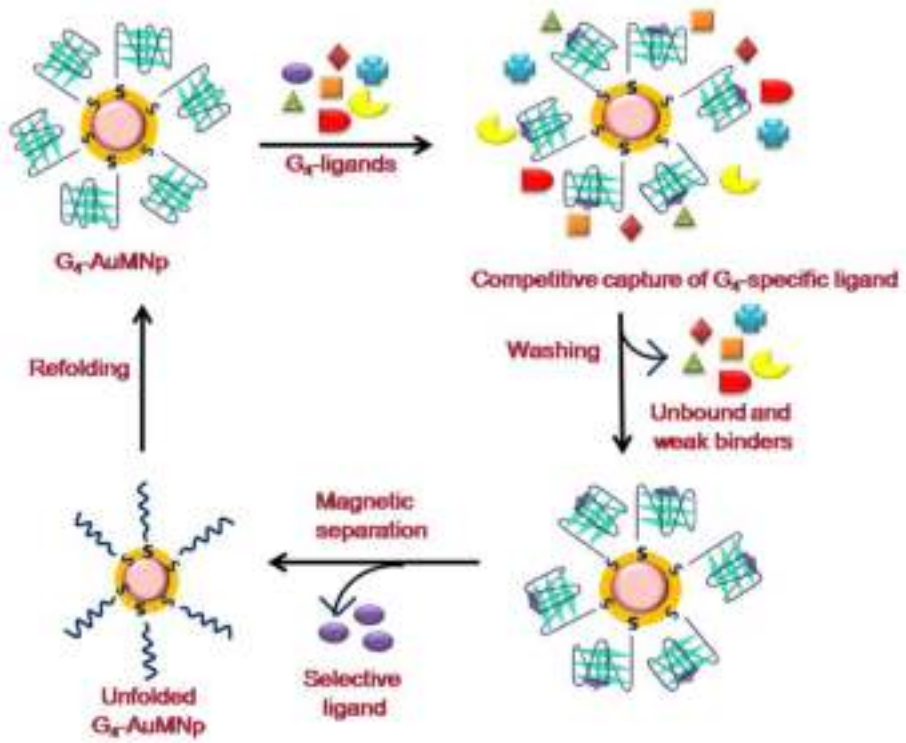


Figure 9. G_4-AuMNps-mediated pull-down-based screening assay.

(4) Target guided Bio-orthogonal synthesis of ligands within cellular systems

Recently, Dash and co-workers demonstrated that DNA G4s can promote macrocyclization-like challenging reactions, enabling the synthesis of molecules specifically designed for modulating gene function (Figure 10).[58] The planar G-quartets present within DNA G4s provided a size complementary reaction platform for the macrocyclization of bifunctional azide and alkyne fragments from a pool of reacting fragments. G-quadruplexes were grafted on magnetic nanoparticles for easy identification of the best binder from the

reaction mixture. The peptidomimetic macrocyclic ligand exhibited excellent binding affinity for G-quadruplexes. The bio-orthogonal in situ click reaction occurred without interfering with the DNA G-quadruplex biomolecules. The resulting macrocycle, displaying inherent fluorescence was utilized to track its cellular localization using a G4 antibody. As the macrocyclization occurred in 48 h and was templated by two G4s (c-MYC and h-TELO), which are overrepresented in HeLa cells, they subsequently conducted the reaction using the corresponding azide and alkynes in living HeLa cells. ESI-MS analysis of cell lysates incubated with bisazide and bisalkyne revealed the formation of macrocycles by endogenous G4s in living cells. The macrocyle along with its unreacted azide demonstrated excellent downregulation of oncogene expression in cancer cells. Thus, the use of bioorthogonal chemistry using bifunctional azide and alkyne fragments enabled selective and controlled synthesis of macrocyclic ligands in a biologically compatible manner. This approach combining the unique properties of DNA G4s and the selective synthesis enabled by biorthogonal chemistry provides new avenues for designing and optimizing anticancer drugs with enhanced efficacy and specificity.

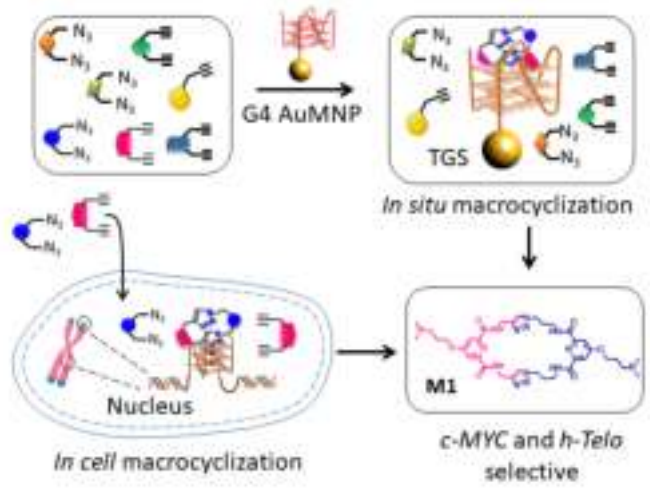


Figure 10. Target guided Bio-orthogonal synthesis of ligands within cellular systems (*Angew. Chem.Int. Ed.* 2023,62, e202215245).

(5) Synthesis of PNA mimics

PNAs are synthetic analogues of natural nucleic acids that can undergo complementary base pairing with themselves as well as DNA or RNA by Watson–Crick base pairing. These molecules can be used for sequence specific targeting of biologically relevant genes, contributing towards genomic alterations. However, the major limitation of PNAs is their poor cellular permeability and water insolubility due to formation of a globular structure. Chemical modification of PNAs can abrogate these inherent shortcomings of PNAs making these molecules as powerful candidates for anticancer therapeutics. Prof. Dash and her group synthesized PNA-like scaffolds by incorporating five-membered thiazole rings as modified bases instead of nucleobases and studied their subsequent effects on gene regulation by biophysical and in vitro assays.[59] A thiazole-modified PNA trimer selectively recognizes c-

MYC G-quadruplex (G4) DNA over other G4s and duplex DNA. It displays a high stabilization potential for the c-MYC G4 DNA and shows remarkable fluorescence enhancement with the c-MYC G4. It is flexible enough to bind at 5′ and 3′ ends as well as in the groove region of c-MYC G4. Furthermore, the PNA trimer easily permeates the cellular membrane and suppresses c-MYC mRNA expression in HeLa cells by targeting the promoter G4.

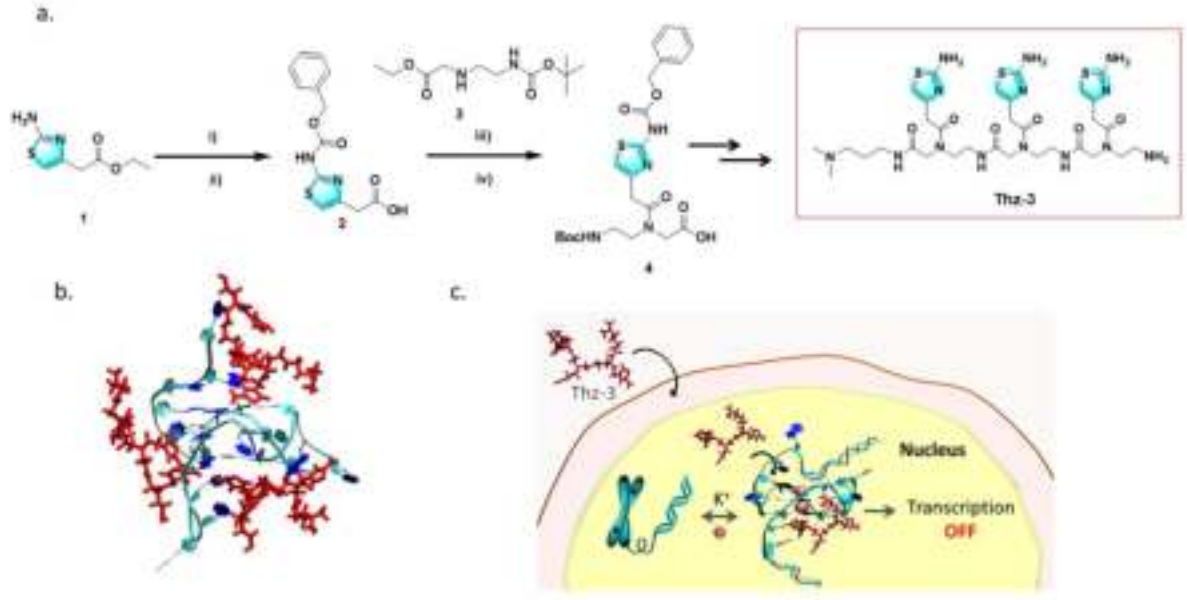


Figure 11. PNA mimics targeting c-MYC G-quadruplex

(B) Bio-inspired functional architectures

DNA secondary structures are not only useful for biomedical applications but also 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. In Prof. Dash's group, they have used DNA secondary structures and their components to construct bio-nanowires, logic gates and 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 12).

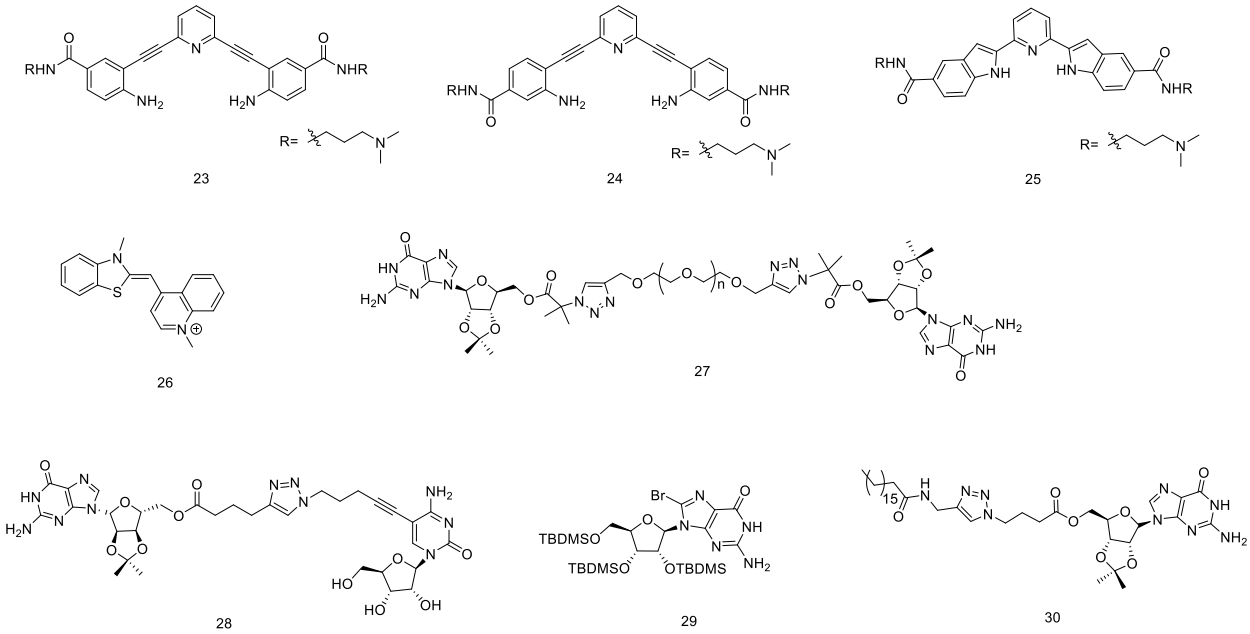


Figure 12. Structure of ligands 23– 30.

(1) Bio-inspired nano-structures:

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

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 high binding affinity towards h-TELO over duplex DNA. Furthermore, the ligand could induce *h-TELO* motif by noncovalent 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.

(2) 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 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 a 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 13). 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 DNaseI as well as performing various logic operations, which may be useful for designing intelligent biomolecular machines.

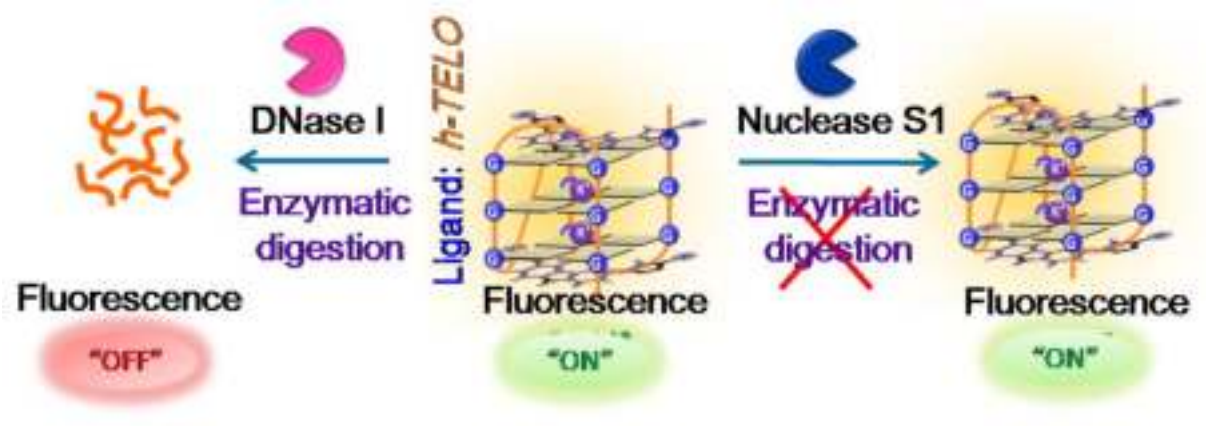


Figure 13. Differential activity of enzymes on ligand bound DNA.

(3) 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 14A). Notably, the conductance of the supramolecular guanosine channels in the phospholipid bilayers has been found to be 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]

In the next 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-cytidinebis-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 14B). It is also noteworthy that the ion channel activity of this bis-nucleoside has been inhibited by the nucleobase cytosine.

In 2018, the self-assembly of a lipophilic tert-butyldimethylsilyl (TBDMS) protected bromo guanosine derivative G1 **(29)** to form different nano-structures 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, development of 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 transportation of potassium ions.

Very recently, the construction of an artificial ionophore using a telomeric DNA G-quadruplex and a lipophilic guanosine (MG) **(30)** has been delineated.[67] Biophysical studies revealed that MG stabilizes *h-TELO* G-quadruplex by non-covalent interactions and its lipophilic chains facilitate the insertion the *h-TELO*G-quadruplex within the lipid bilayer to form the ionophore (Figure 14C). 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.

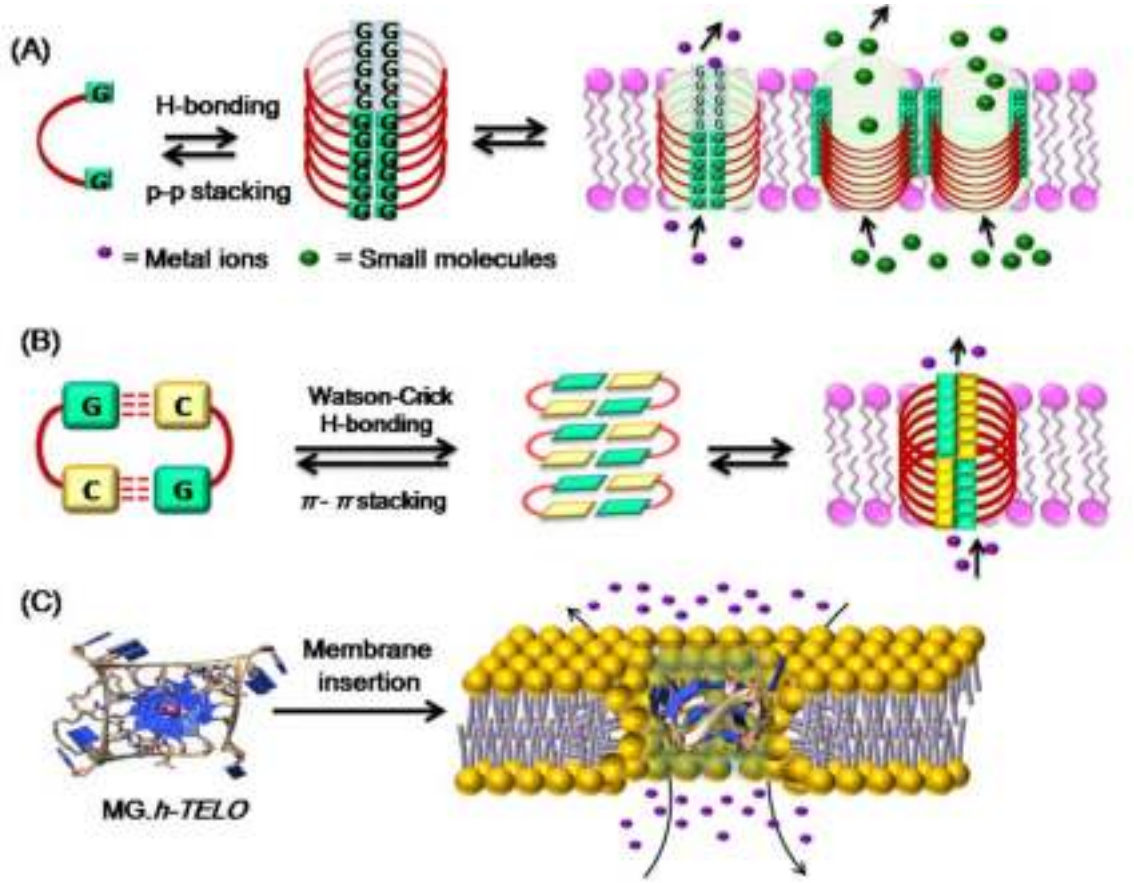


Figure 14. Artificial ion channels based on nucleoside derivatives.

(4) 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 15A). The K^+ stabilized hydrogel binds to iron (III)-hemin and shows DNAzyme like peroxidase activity, catalyzing 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 and this conformational switching has been used to devise a molecular logic gate for sensing of 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 15B). These hydrogels, presumably formed by an i-motif like arrangement of cytidine and its boronate ester analogues, possess excellent thixotropic and self-healing properties. Moreover, these hydrogels show potent antibacterial activities against various Gram-negative bacteria. In addition, these supramolecular metallogel was also capable of inducing apoptotic-like cell death of protozoan parasite *Leishmania major* by causing damage to the membrane as well as DNA. These hydrogels could therefore find promising applications in combating cutaneous leishmaniasis by topical treatment.[72]

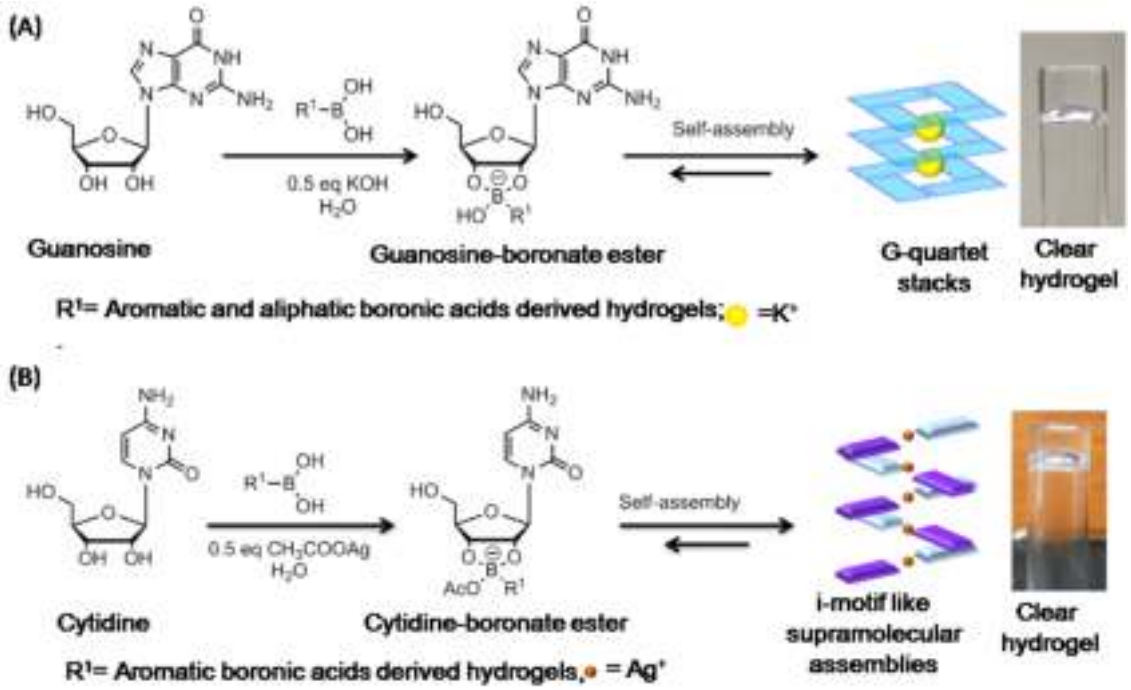


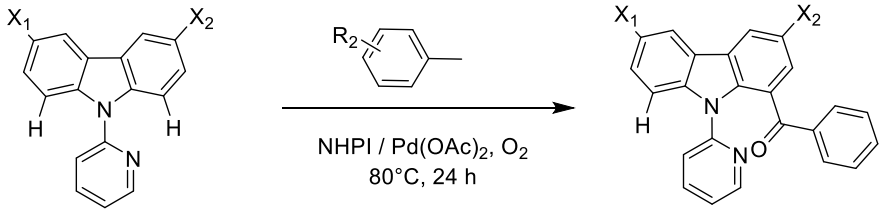
Figure 15. Nucleoside derived supramolecular hydrogels.

(C) Synthesis of biologically active compounds:

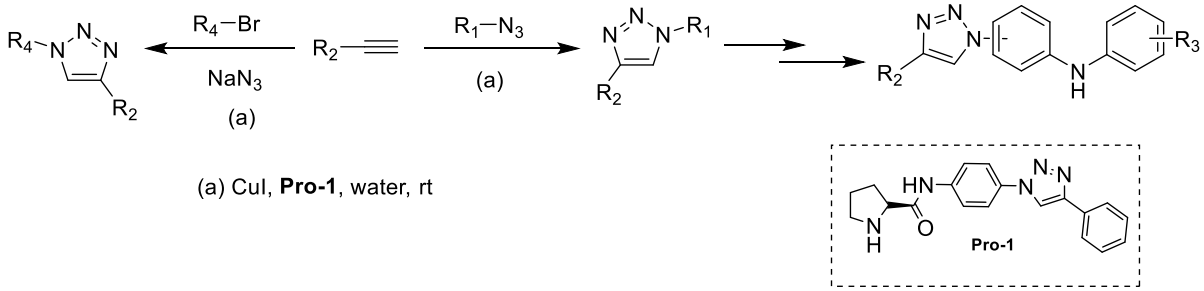
The most significant contributions of Prof. Dash in this area are development of a) new approaches like ring-closing metathesis, diversity-oriented synthesis for heterocyclic ring systems and naturally occurring carbazole alkaloids, b) supported/organocatalytic methods to

synthesize enantiopure molecules including tubavaline core of the natural product tubulysine, a potent anticancer agent and c) sustainable procedures involving transition metal free and uncatalyzed reactions. These methods may find industrial applications for the synthesis of natural products and complex organic molecules. Some of the contributions of Prof. Dash in this field are as follows.

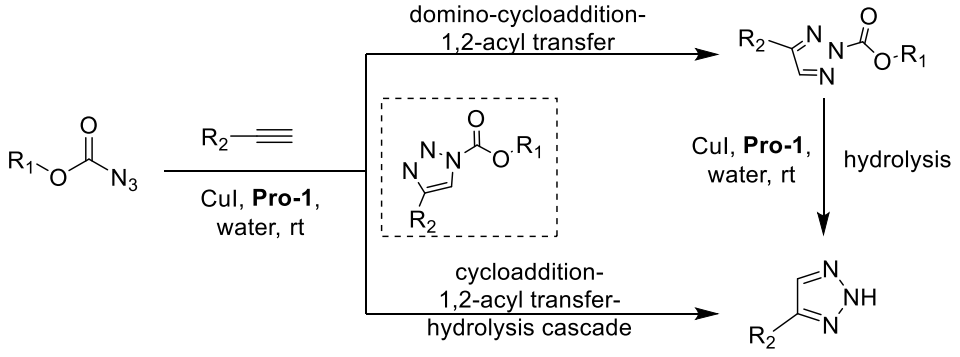
1. A palladium-catalyzed monoacylation of carbazoles using toluene derivatives, in presence of NHPI as the cocatalyst and oxygen as the oxidant has been delineated. The acylation of monosubstituted N-pyridylcarbazoles using aldehydes, takes place regioselectively at the C-8 position. This highly site-selective acylation proceeds through a radical process.[73]



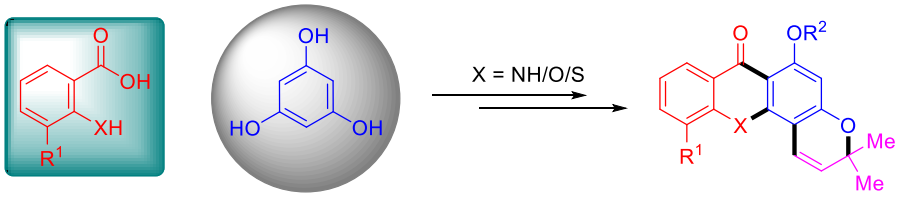
2. Cycloaddition of a wide range of azides with terminal alkynes using catalytic CuI and a prolinamide ligand (**Pro-I**) in aqueous media under aerobic conditions, leading to triazoles formation, has been achieved. The synthetic utility of the method is demonstrated by the synthesis of pharmacologically relevant triazolyl diaryl amines via a Cu(I) catalyzed relay cycloaddition and Ullmann coupling sequence.[74]



3. A cascade sequence involving [3+2] cycloaddition, 1,2-acyl migration and hydrolysis yielding 2H-1,2,3-triazoles via the regioselective formation of N^2 -carboxyalkylated triazoles, has been achieved in aqueous media. The reaction proceeds in presence of a CuI–prolinamide catalyst system, where prolinamide promotes the novel organocatalytic 1,2-acyl migration as well as hydrolysis of the resulting N^2 -carboxyalkylated triazoles.[75]



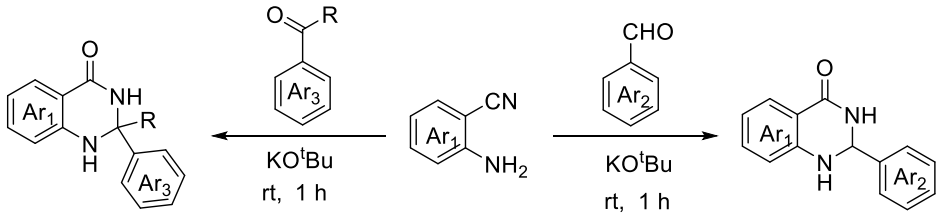
4. The tetracyclic acridone alkaloids acronycines, noracronycines, atalaphyllidine and 5-hydroxynoracronycine are synthesized from commercially available anthranilic acid and phenol derivatives via condensation reaction and regioselective annulation in excellent overall yields and in minimal steps. Moreover, the present strategy has been showcased in the synthesis of oxa and thia analogues of acronycine alkaloid.[76]



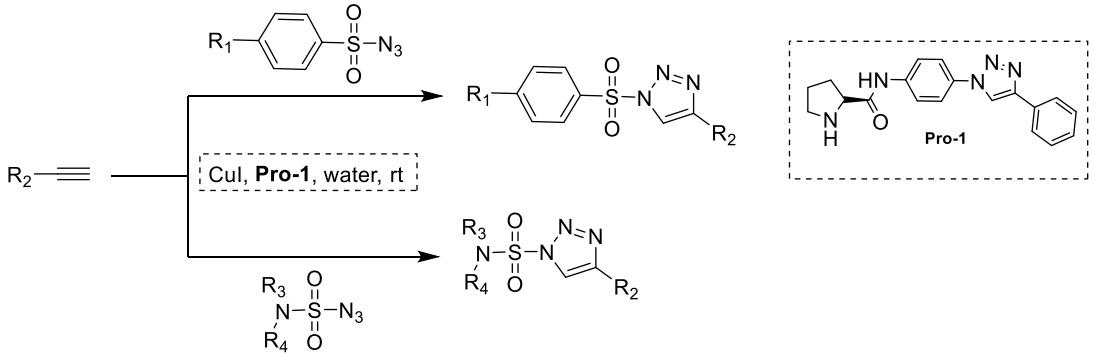
- *simple and scalable synthetic pathway*
- *regioslective annulation*
- *synthesis of tetracyclic acridone alkloids*
- *excellent yields*
- *synthesis of oxa and thia analogues of acridone alkaloids*

X = NMe, R^1 = H, R^2 = Me; Acronycine
X = NMe, R^1 = H, R^2 = H; Noracronycine
X = NH, R^1 = OH, R^2 = H; Atalaphyllidine
X = NMe, R^1 = OH, R^2 = H; 5-hydroxynoracronycine

5. An efficient synthetic protocol to access heterocyclic dihydroquinazolinones by a transition-metal free process, involving the reaction of 2-aminobenzonitriles with aldehydes in the presence of KO^{t}Bu has been achieved. Following a radical pathway, the reaction is feasible at room temperature, features a broad substrate scope and tolerance to a wide range of functional groups.[77]

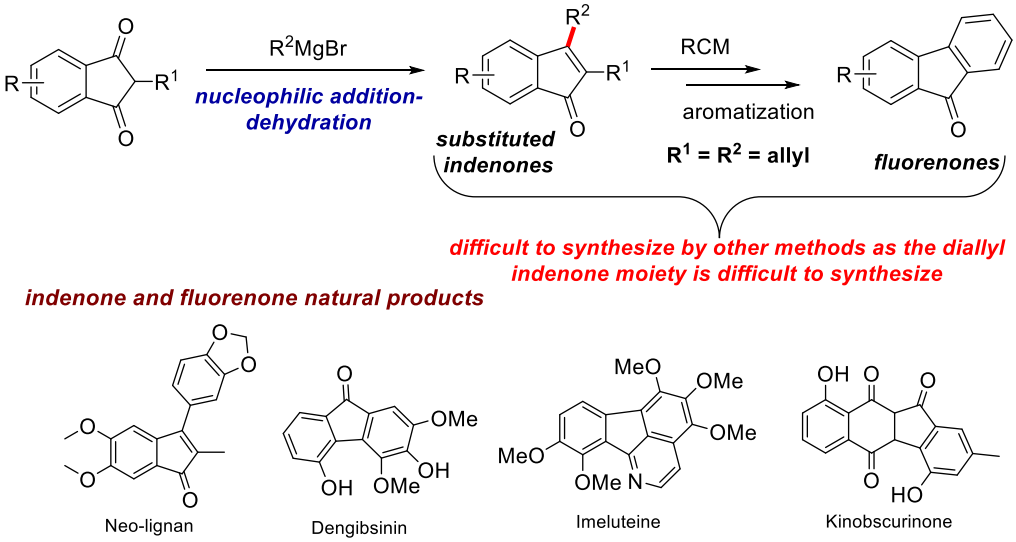


6. A Cu(I) catalyzed method using a prolinamide ligand that selectively generates N-sulfonyl and sulfamoyltriazoles in aqueous media by inhibiting the cleavage of the N1–N2 bond of 5-cuprated triazole intermediates, has been delineated. The method is mild and tolerant to air, moisture and a wide range of functional groups, thereby providing easy access to a variety of triazole products.[78]

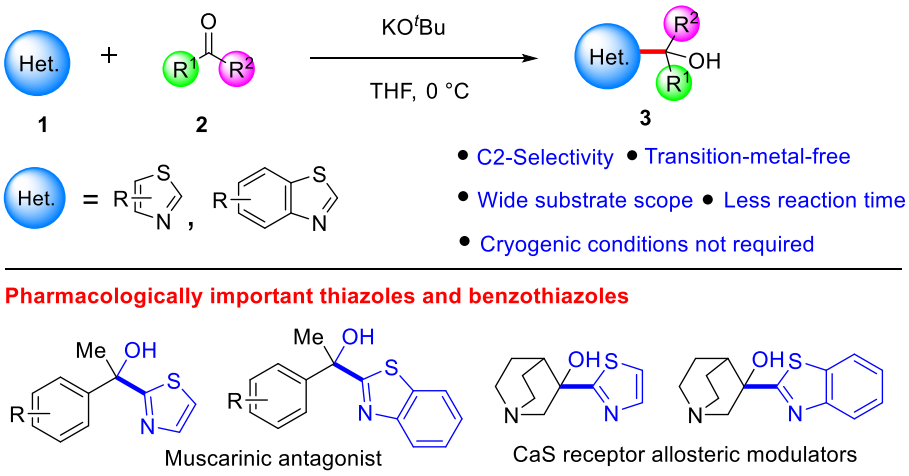


7. An efficient, one-step method has been used to access substituted indenones derivatives and natural products such as neo-lignan and isoampelopsin D, in a regiospecific manner by

Grignard addition to indene-1,3-dione. Additionally, the synthesized diallyl indenones serve as RCM precursors, yielding substituted fluorenone derivatives via RCM-aromatization sequence.[79]



8. A transition metal-free direct C-2 hydroxymethylation of thiazoles and benzothiazoles in the presence of KO^{t}Bu, has been reported. Selective deprotonation drives Csp2-Csp3 bond formation thus creating a quaternary center. This method complements traditional azole halide cross-coupling, which also enables multigram synthesis of thiazolyl diarylcarbinols and bioactive compounds.[80]



Non-canonical nucleic acids have emerged as attractive targets as well as tools in anticancer drug development. Prof. Dash has employed various chemical approaches for designing and synthesizing selective ligands against a particular G-quadruplex or i-motif structure. Recently reported molecules can further be explored for preclinical and clinical trials and may be used as potential therapeutic candidates for the treatment of human cancers. In addition, she has 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 by Prof. Dash will undoubtedly lead to many new opportunities and innovative applications for developing next generation therapeutics and nanodevices.

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Signature of Jyotirmayee Dash