

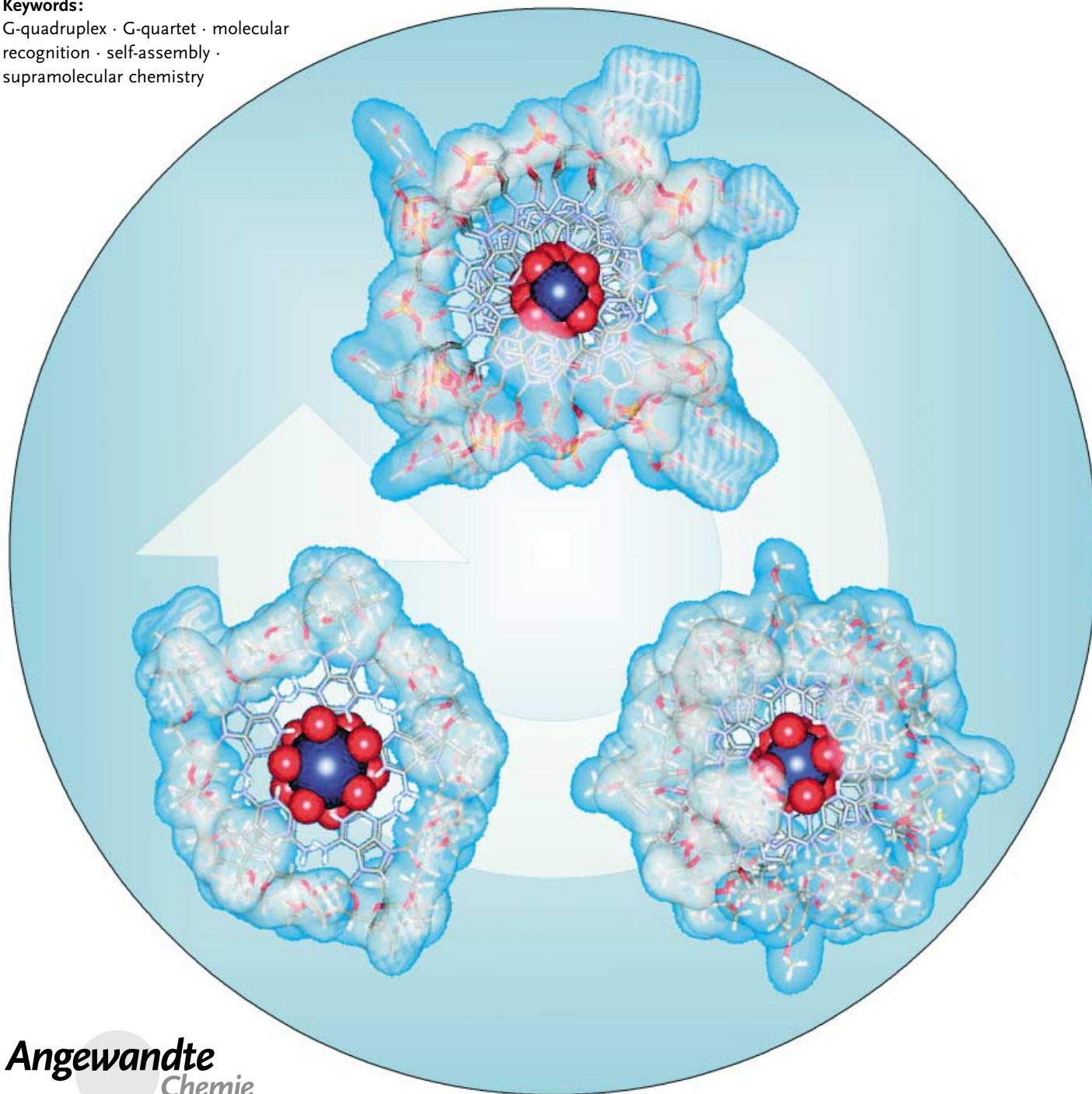
Supramolecular Chemistry

G-Quartets 40 Years Later: From 5'-GMP to Molecular Biology and Supramolecular Chemistry

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G-quadruplex · G-quartet · molecular recognition · self-assembly · supramolecular chemistry



Molecular self-assembly is central to many processes in both biology and supramolecular chemistry. The G-quartet, a hydrogen-bonded macrocycle formed by cation-templated assembly of guanosine, was first identified in 1962 as the basis for the aggregation of 5'-guanosine monophosphate. We now know that many nucleosides, oligonucleotides, and synthetic derivatives form a rich array of functional G-quartets. The G-quartet surfaces in areas ranging from structural biology and medicinal chemistry to supramolecular chemistry and nanotechnology. This Review integrates and summarizes knowledge gained from these different areas, with emphasis on G-quartet structure, function, and molecular recognition.

1. Introduction

In 1990 Guschlbauer, Chantot, and Thiele published the review article "Four-Stranded Nucleic Acid Structures 25 Years Later: From Guanosine Gels to Telomer DNA."^[1] In that paper, they pointed out the emerging importance of the G-quartet, a hydrogen-bonded ionophore first identified in 1962 (Figure 1).^[2] Renewed attention to the G-quartet in the late 1980s was generated by intriguing proposals that the motif, when formed in DNA, might be biologically relevant.^[3,4] Today, interest in G-quartet structures remains unabated. Thousands of reports on some aspect of G-quartet structure or function have since appeared, including some

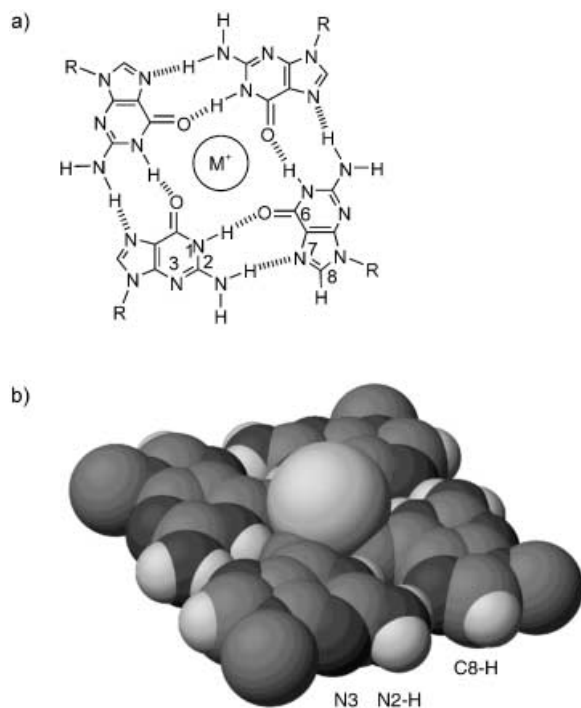


Figure 1. a) The G-quartet; b) space-filling model from the crystal structure of $[1]_{16} \cdot 3K^+ / Cs^+ \cdot 4pic^-$ showing a G-quartet with a K^+ ion bound above the planar assembly.^[29] The riboses have been removed for clarity.

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excellent reviews.^[5–14] G-quartet structures now surface in areas ranging from structural biology and medicinal chemistry to supramolecular chemistry and nanotechnology.

The G-quartet, a hydrogen-bonded complex that binds cations, fits particularly well with contemporary studies in molecular self-assembly and noncovalent synthesis.^[15–26] The activities in molecular self-assembly can be divided into three areas: 1) biomimetic studies, 2) basic studies of noncovalent interactions, and 3) synthesis of new assemblies; Figure 2 indicates the connections between these divisions. Molecular self-assembly is a hallmark of many natural and synthetic systems and good models for understanding self-assembly often come from inspecting nature (pathway A in Figure 2).^[27] A model aims to simplify a complex system, thus allowing one to focus on specific interactions that are important for the self-assembly (B). Fundamental information from model studies may be used to either better

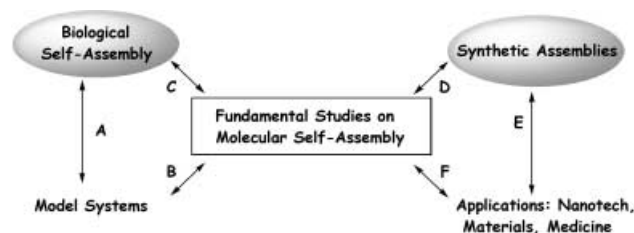


Figure 2. Noncovalent interactions are central to both biological self-assembly and to the synthesis of new supramolecular structures.

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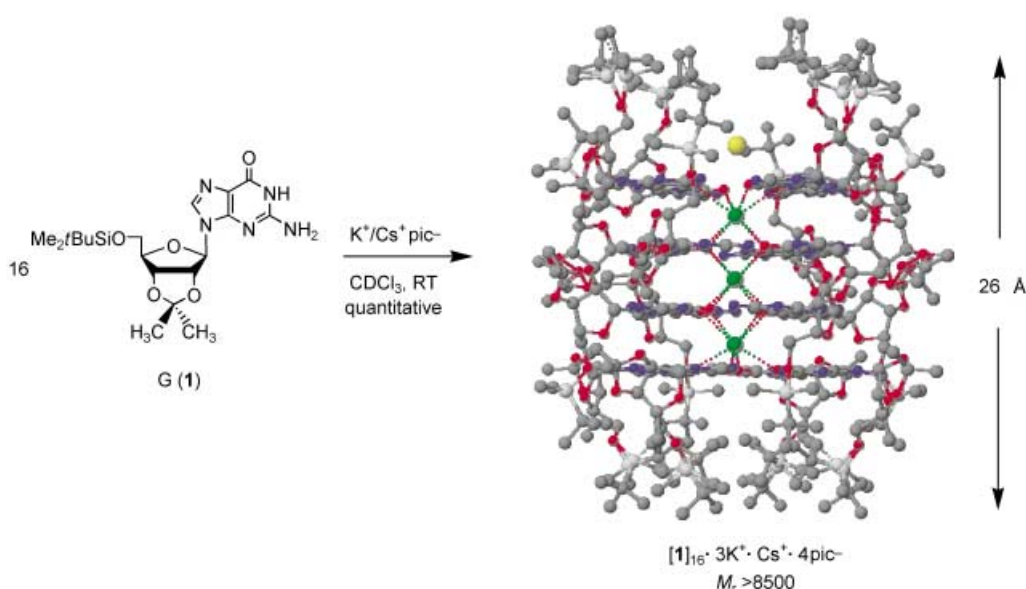


Figure 3. The cation-templated self-assembly of 16 equivalents of 5'-silyl-2',3'-O-isopropylidene guanosine (**G, 1**) gives a lipophilic G-quadruplex $[1]_{16} \cdot 3K^+ \cdot Cs^+ \cdot 4pic^-$ in the solid state and in solution (green K, yellow Cs, blue N, red O).^[29] This G-quadruplex, with four stacked G-quartets, is prepared quantitatively by extracting salts from water with a solution of **1** in $CHCl_3$.

understand the parent system (C) or to build and apply new synthetic systems (D–F).^[27,28] As discussed below, the G-quartet serves as an excellent model to learn about the fundamentals and utility of molecular self-assembly.

1.1. An Introductory Example: Stabilization of Lipophilic G-Quadruplexes by Noncovalent Interactions

An example from our research introduces some of the key structural features of the G-quartet and also highlights the different noncovalent interactions that drive the self-assembly of guanosine. In the presence of templating cations 5'-silyl-2',3'-O-isopropylidene guanosine (**G, 1**) forms a lipophilic G-quadruplex that is stable in nonpolar solvents and that also gives single crystals with long-range order. A G-quadruplex is defined as a structure built from the vertical stacking of multiple G-quartets. Over the past few years Fettinger and co-

workers have solved the crystal structures of a number of lipophilic G-quadruplexes.^[29–33] The G-quadruplex depicted in Figure 3, with a formula of $[1]_{16} \cdot 3K^+ \cdot Cs^+ \cdot 4pic^-$, illustrates the synthetic potential of self-assembly. Upon mixing in an organic solvent, 24 components organize into a single diastereomer with dimensions of $26 \times 30 \times 30 \text{ \AA}$ and a molecular weight of over 8500.^[29] The lipophilic G-quadruplex $[1]_{16} \cdot 4M^+ \cdot 4pic^-$ is also functional: it is a self-assembled ionophore that can extract salts from water into organic solvents.

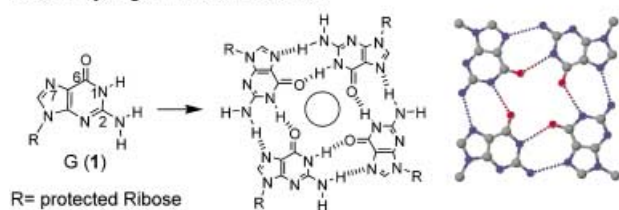
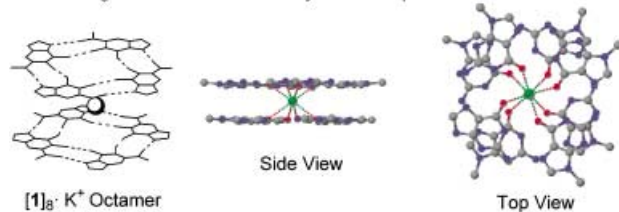
While the noncovalent interactions that enable formation of a G-quadruplex are certainly interconnected, the structure can be dissected into three organizational levels (Figure 4). In the first level, four molecules of **G(1)** use self-complementary hydrogen bonds to form a planar G-quartet. As shown in level II, the G-quartets stack with a separation of 3.3 \AA between individual layers. An octacoordinate cation, which forms cation–dipole interactions with eight separate molecules of **1** and located between two G-quartets, stabilizes hydrogen-bonded quartets and enhances base-stacking interactions to provide a C_4 -symmetric $G_8 \cdot K^+$ octamer. In level III, four picrate anions hydrogen bond to amino groups projecting from the G-quartets. These nucleobase–anion hydrogen bonds link the two inner G-quartets in the D_4 -symmetric hexadecamer $[1]_{16} \cdot 4M^+ \cdot 4pic^-$, a structure that is stable in the solid state and solution.^[29–32] One remarkable feature of this structure is the four colinear cations, spaced 3.3 \AA apart, that are arranged down a central channel. The electrostatic repulsion that might be expected between the channel cations is clearly minimized by the G-quartet oxygen atoms and aromatic rings.

There is also a stereochemical consequence to the cation-templated self-assembly of **1**. A G-quartet with chiral sugars attached to each nucleobase has two diastereotopic faces: a



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Level I. Hydrogen-Bonded G-Quartet


 Level II. $G_8 \cdot M^+$ Octamer Formed by Cation–Dipole Interactions and π Stacking


Level III. Hexadecameric G-quadruplex with Anion–Nucleobase H-Bonds

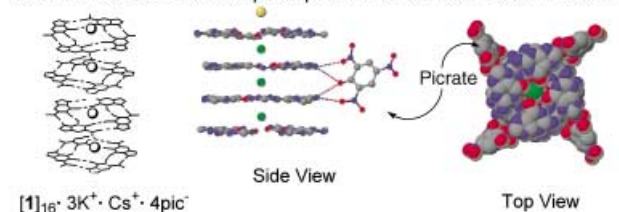


Figure 4. Schematic representation of hierarchical self-assembly processes that occur to give the lipophilic G-quadruplex [1]₁₆·3K⁺/Cs⁺·4pic⁻.^[29] The structure is held together by nucleobase hydrogen bonds, cation–dipole interactions, stacking interactions, and hydrogen bonds between the nucleobase and anion. The ribose units have been omitted for clarity.

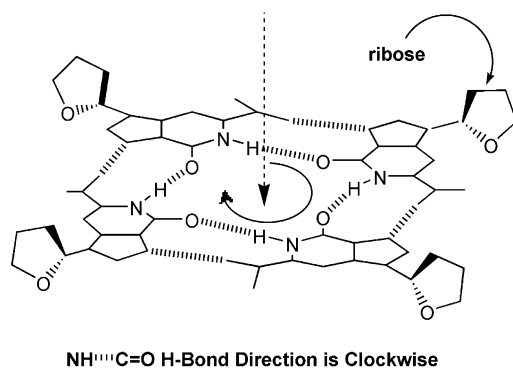


Figure 5. The attached sugars result in the chiral G-quartet having diastereotopic faces, a “head” and a “tail”. The “head” of the G-quartet is that face with clockwise rotation of the NH to C=O hydrogen bonds.^[34]

“head” and a “tail” (Figure 5).^[34] In this way the nucleoside sugars transfer and amplify their chirality upon the supramolecular organization of chiral G-quartets. Although there are 16 stacking permutations for a 4-layered G-quadruplex, only a single diastereomer of [1]₁₆·4M⁺·4pic⁻ is observed.^[29] This selectivity for this noncovalent assembly of formula [1]₁₆·4M⁺·4pic⁻ is striking since, unlike in DNA, there is no covalent backbone connecting the G subunits to induce supramolecular helicity. Various noncovalent interactions

(hydrogen bonds, cation–dipole interactions, and van der Waals interactions) work together to fix the structure and stereochemistry of these lipophilic G-quadruplexes. The cooperativity of these noncovalent forces is a hallmark of guanosine self-assembly in biology and synthetic chemistry.

1.2. The Review's Organization

After this Introduction, a review of early studies on guanosine self-association is given in Section 2. This work provided the foundation for later structural biology and supramolecular studies. Section 3 discusses G-quadruplexes in molecular biology and medicinal chemistry. In particular, structural and recognition studies have been highlighted that might appeal to chemists looking to learn about self-assembly from nature. Section 4 gives a review of the self-assembly of lipophilic nucleosides. Here, assemblies that are models for DNA structure and that also provide inspiration for new supramolecular forms and functions are discussed. Section 5 focuses on applications of guanosine self-association in materials science and nanotechnology. Section 6 provides a summary and an outlook.

2. The Early Studies on the Self-Assembly of 5'-Guanosine Monophosphate

The self-association of guanine occurs in many settings. Some spiders have cells known as guanocytes that are filled with crystalline plates of guanine (Figure 6). When disturbed, these spiders change color instantly by retracting the guanocytes from their surface.^[35] The eyes of certain deep-sea fish contain layered guanine crystals that focus light to the photoreceptors.^[36]

Guanosine derivatives are notoriously intractable in the laboratory.^[37] The review by Guschlbauer et al. tabulated 30 different G derivatives that gel in water.^[1] It is not surprising that guanosine self-associates: its edges have self-complementary hydrogen-bond donors and acceptors, and its polarizable aromatic surface, with a strong molecular dipole,^[38] is ideal for stacking. This basis for self-association was established over 40 years ago, when Gellert et al. reported that 3'-GMP (2) and 5'-GMP (3) form layers of hydrogen-bonded tetramers.^[2] Their model, developed from fiber diffraction studies, had the N1-H and N2-H donor atoms of one guanine molecule pairing with the N7 and O6 atoms of a neighboring guanine molecule. The resulting G-quartet is a planar macrocycle held together by eight hydrogen bonds. The authors proposed that G-quartets stacked 3.25 Å apart formed a helix, which was consistent with later diffraction data obtained for guanosine analogues and polyguanylic acid.^[39–41] Shortly after the report by Gellert et al., Fresco and Massoulié reported that polyguanylic acid also formed a multistranded helix in solution.^[42] They, too, correctly proposed that stacking hydrogen-bonded G-quartets stabilized the polyguanylate assemblies.

In the 1970s and 1980s two key discoveries were made concerning the self-association of guanosine. First, 5'-GMP

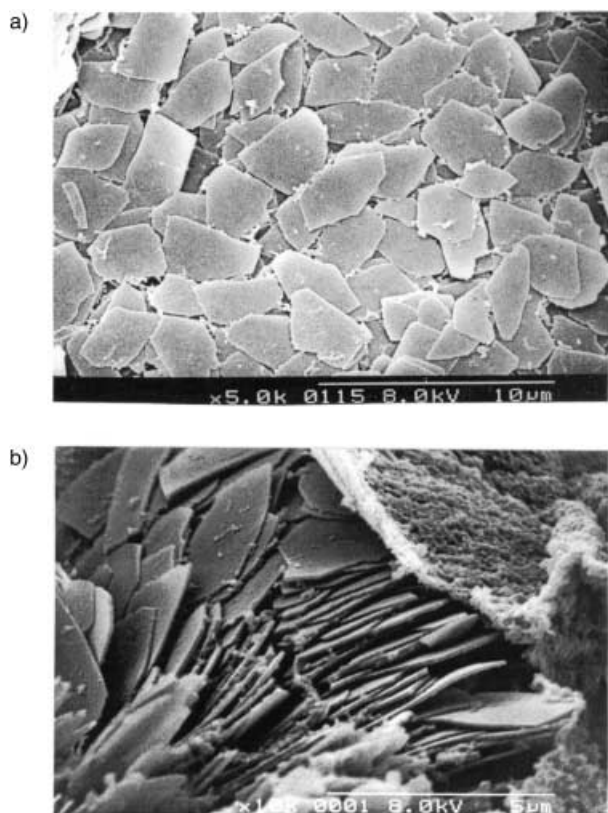
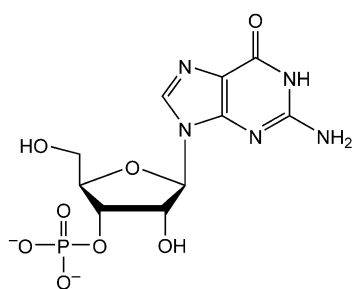
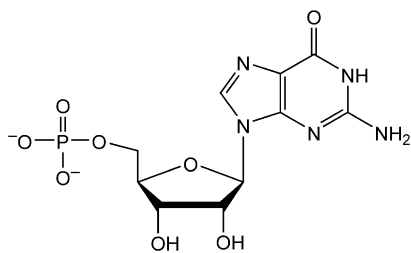


Figure 6. Crystalline guanine deposits from the spider *Tetragnatha polychromata*.^[35b] a) Surface view of crystalline guanine plates (approximately $4 \times 2 \mu\text{m}$). b) Side view of the same crystals showing the thinness and stacking of the guanine plates. The photographs were supplied by Harriet Mitchell.



3'-GMP (2)



5'-GMP (3)

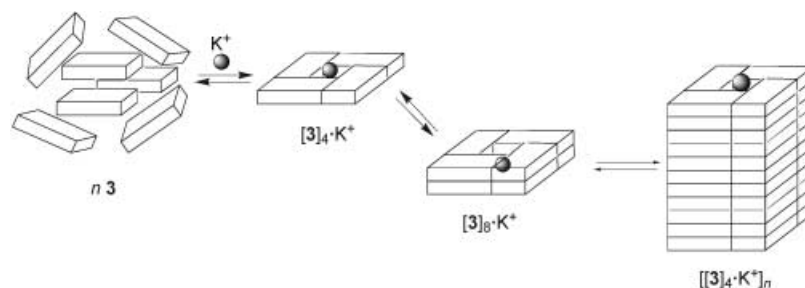


Figure 7. Equilibria involved in the self-association of 5'-GMP (3). Poorly defined aggregates are formed in the absence of cations. Cations template the formation of the planar G-quartets, which can give discrete $[3]_8 \cdot \text{M}^+$ octamers or helical G-quadruplex stacks.

(3) did not always give gels. At basic pH values, where it is dianionic, 5'-GMP (3) formed smaller assemblies that could be studied by NMR spectroscopy.^[43] Second, alkali-metal cations, particularly K^+ and Na^+ , stabilized these complexes.^[44] Cation binding is understandable, since a G-quartet has four oxygen atoms clustered in its center. Without a bound cation, this cyclic arrangement would be electronically unfavorable,^[45] and so, the cation is essential for templation and stabilization of the G-quartet. Discrete cation-bound octamers of the type $\text{G}_8 \cdot \text{M}^+$ were proposed to be the building blocks for extended columns of stacked G-quartets (Figure 7). Despite different conclusions about stoichiometry and stereochemistry,^[46,47] it was clear that G-quartets were the foundation for the self-assembly of guanosine. The basics of guanosine self-assembly were confirmed by dynamic light scattering, single-crystal X-ray, electrospray mass spectrometric, and solid-state NMR spectroscopic studies.^[48–53]

These early G-quartet studies remain timely, as they are pertinent to themes that surround supramolecular chemistry today. For example, dynamic combinatorial chemistry is a powerful method for generating molecular receptors.^[54–57] The findings that 5'-GMP (3) self-associates only with certain cations^[44] and that G-C base pairs give way to G-quartets upon addition of K^+ ions^[58] exemplify the shift in equilibrium that is the basis of dynamic combinatorial chemistry. Whereas template experiments with Li^+ and Cs^+ ions showed no effect, the addition of Na^+ or K^+ ions to 5'-GMP (3) gave G-quartet-based structures, and prompted the conclusion: “We believe this to be the first demonstration of the ability of alkali metal ions to direct structure formation of a nucleotide through a size-selective coordination mechanism”.^[44] Pinnavaia et al. suggested that K^+ ions were too large to be coplanar and fit

between G-quartets to give a $\text{G}_8 \cdot \text{K}^+$ octamer. The estimated K–O distance in the octamer matched bond lengths for known octacoordinate K^+ complexes. The authors summarized this key feature: “We suggest that cavity complexation as described contributes stability to the complex, and that it is a necessary condition for self-structure formation by neutral GMP”.^[44] Crystal structures of the lipophilic G-quadruplex $[\text{I}]_{16} \cdot 3 \text{K}^+$ and of the DNA G-quadruplex $[\text{d}(\text{GGGGTTTGGGG})_4]$ later showed K^+ ions sandwiched between G-quartets.^[29,59,60]

The NMR spectrum of the assembly also raised issues about supramolecular stereochemistry, an area intensely studied today.^[61–63] Stacking two chiral G-quartets can give at least six possible diastereomers for a $G_8 \cdot M^+$ octamer, depending on the stacking orientation and relative rotation about the central axis.^[46] The NMR spectra for $5'$ -GMP·Na⁺ indicated, however, the presence of only two stable diastereomers, thus indicating a high stereoselectivity in this self-assembly process.

Another important finding concerned the stability of the assembly towards dissociation. The ^1H NMR spectra of $5'$ -GMP·Na⁺ showed separate signals for the monomer and the assembly.^[43,44] This slow exchange was remarkable since such kinetically stable systems are rare, especially in water. Hydrogen-bonded assemblies and their components are usually in fast exchange and give averaged NMR signals. The self-assembly of $5'$ -GMP **3** is also relevant today, since obtaining discrete noncovalent assemblies that are thermodynamically and kinetically stable in water is still challenging.^[64–67]

Another topical area of supramolecular chemistry involves the mechanisms of assembly and disassembly.^[68] Again, the early studies with GMP were also concerned with such issues. Multinuclear NMR studies by Laszlo, Detellier, and co-workers were especially insightful.^[47,69,70] Analysis by ^{23}Na NMR spectroscopy revealed that the Na⁺ ions moved in and out of the $[3]_8 \cdot \text{Na}^+$ octamer 10^4 – 10^8 times per second, which is orders of magnitude faster than ligand exchange.^[69] These assemblies did not need to fully dissociate to release their guests, which suggests that such systems might be useful for mediating cation exchange. The ^1H NMR data also provided thermodynamic constants for the formation of the octamer $[3]_8 \cdot \text{K}^+$ ($\Delta H = -17 \pm 2 \text{ kcal mol}^{-1}$ and $\Delta S = -51 \pm 6 \text{ cal mol}^{-1} \text{ K}^{-1}$) that indicated an enthalpic process: “Self-assembly of the $5'$ -GMP nucleotides does not occur because of release of water. Self-assembly of $5'$ -GMP, unlike a normal stacking interaction is not determined predominantly by hydrophobic forces. Cation binding plays an important role, together with and reinforcing the hydrogen bonding of the guanines into planar tetramers.”^[47]

Finally, one active area in supramolecular chemistry involves the self-organization of noncovalent assemblies, which is important in materials science and nanoscience.^[71] The G-quartet system based on $5'$ -GMP (**3**) provides a nice example of this self-assembly/self-organization paradigm.^[72–74] It was shown that GMP oligomers—from mononucleotide $5'$ -GMP **3** to the d(GGGGGG) hexanucleotide—formed liquid crystals in water (Figure 8).^[72,73] The CD and X-ray diffraction data of these oligomers were consistent with the formation of helical rods containing stacked G-quartets. At increasing concentration these compounds formed a hexagonal liquid crystalline phase by lateral association of G columns (Figure 8c). The finding that self-assembled G-quadruplexes could self-organize into liquid crystals with long range order was important for later applications in materials science and nanotechnology (see Section 5).

In retrospect, these early studies on the self-association of $5'$ -GMP (**3**) involved much of what motivates studies in supramolecular chemistry today, namely structure and stereo-

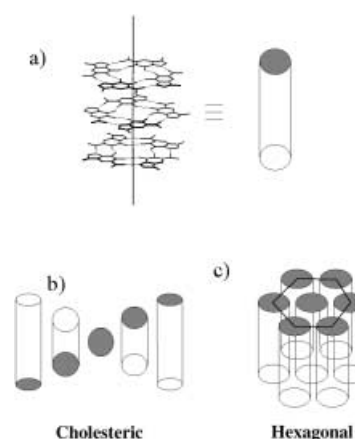


Figure 8. Formation of liquid crystals by G derivatives. a) G-quartets stack to give a columnar structure. b) The relative arrangements of the G-quartet columns in the cholesteric phase and c) in the hexagonal liquid-crystalline phase. Reprinted in a modified form from ref. [7]

chemistry, mechanism, and function. However, recognition of the name G-quartet really developed in the late 1980s, when it was proposed that G-quartets might be biologically functional.^[3,4,75,76] In particular, Sen and Gilbert suggested that G-quartets were involved in chromosome association during meiosis.^[75] Sundquist and Klug proposed that the G-quadruplex was an important secondary structure that regulated biochemical processes in the telomeric region of the chromosome.^[76] A research explosion on G-quadruplexes followed.

3. Nucleic Acid G-Quadruplexes: Structure and Molecular Recognition

G-quadruplexes are formed in vitro by DNA and RNA oligonucleotides with sequences that occur in chromosomal telomeres, gene promoter regions, recombination sites, RNA packaging sites, and RNA dimerization domains.^[12–14] For many years there has been debate as to whether these structures have any functional roles in living cells. Many papers contain the qualifier that the role of the G-quartet in vivo is yet to be proven, but evidence is now mounting that DNA and RNA G-quadruplex structures do indeed have in vivo relevance. For example, the most common form of mental retardation, fragile X syndrome, is caused by a mutation to the fragile X mental retardation protein (FMRP), which transports messenger RNA. One theory is that fragile X syndrome results when mutant FMRP doesn't properly bind mRNA.^[77] Darnell et al. recently discovered that FMRP binds particularly tightly to G-quadruplex RNA.^[78a] They concluded that... “(T)hese data demonstrate that G quartets serve as physiologically relevant targets for FMRP and identify mRNAs whose dysregulation may underlie human mental retardation.” In an accompanying study, Warren and co-workers used antibodies to pull out FMRP and its bound RNAs from mouse brains.^[78b] Seventy percent of the several hundred mRNAs identified were proposed to form the RNA G-quadruplex.

Other major developments occurred in 2002 when Hurley and co-workers reported *in vivo* evidence for the structure and function of G-quadruplex in a gene-promoter region within human cells.^[79] This intramolecular G-quadruplex, made up of a 20–30 base-pair region, was specifically targeted and stabilized by a small molecule, which resulted in transcription repression of the oncogenic *c-myc* protein. Furthermore, Riou et al. reported that another small molecule designed to stabilize G-quadruplex DNA impairs telomerase activity in cancer cells.^[80] Reports of controlling gene expression and *in vivo* enzyme activity by targeting G-quadruplexes will certainly fuel more investigations into characterizing and controlling these nucleic acid structures.

3.1. Overview of Oligonucleotide G-Quadruplex Structure

In the past decade there have been many NMR studies and a growing number of X-ray structures of G-quadruplexes (see Table 1), and the topic of structure has been reviewed in many articles.^[8–10,13]

Oligonucleotide G-quadruplexes differ in their chain number and orientation (Figure 9). Quadruplexes are always stabilized by cations and can form from four separate strands, from two pieces, or from a single chain. NMR spectroscopic analysis has shown that oligonucleotides with a single sequence of G residues form quadruplexes with four parallel strands (Figure 9a).^[81–82] A crystal structure of $[d(TGGGGT)]_4$ with bound Na^+ ions is shown in Figure 10.^[83,84] Two separate helices, each containing four G-

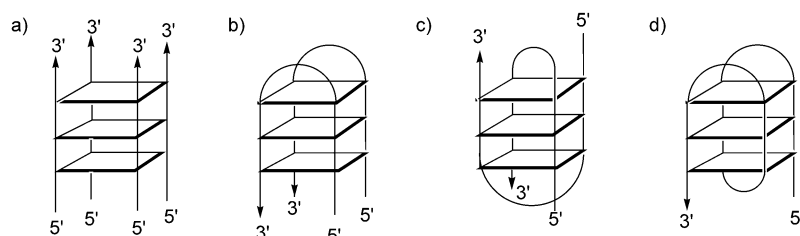


Figure 9. Different G-quadruplex DNA structures. a) A parallel stranded tetraplex; b) a bimolecular complex formed from hairpin dimerization with “edgewise” loops; c) a bimolecular complex with “diagonal” loops; and d) a unimolecular G-quadruplex.

Table 1: X-ray crystal Structures of G-Quadruplexes.

Structure	Comment	Year	PDB entry	Ref.
<i>Oxytricha</i> $d(G_4T_4G_4)_2$	K^+ -bound bimolecular G-quadruplex protein–DNA complex	1992	1D59	[91]
thrombin binding aptamer and thrombin		1993	1HUT	[129]
		1996	1HAP	[130]
$d(TGGGGT)$ with Na^+	parallel four-stranded G-quadruplex	1994	244D	[83]
		1997	352D	[84]
		2000		[29]
$[1]_{16} \cdot 3K^+ / Cs^+ \cdot 4pic^-$	first lipophilic G-quadruplex	2000		[29]
$r(UGGGGU)_4$ with Sr^{2+}	RNA G-quadruplex with base octad	2001	1J8G	[126]
$d(G_4T_4G_4)_2$ and <i>Oxytricha</i> telomere binding protein	DNA–protein complex with bimolecular G-quadruplex	2001	1JB7	[59]
human telomeric DNA sequences	parallel bimolecular G-quadruplex with fold-back loops	2002	1K8P	[95]
<i>Oxytricha</i> $d(G_4T_4G_4)_2$		K^+ -bound bimolecular quadruplex	2002	1JPQ
			1JRN	
<i>Oxytricha</i> $d(G_4T_4G_4)_2$ with 9	first DNA-quadruplex-drug complex		2003	1L1H
$d(TGGGGT)_4$ with daunomycin trimer	DNA-quadruplex-drug complex	2003	1O0K	[191]

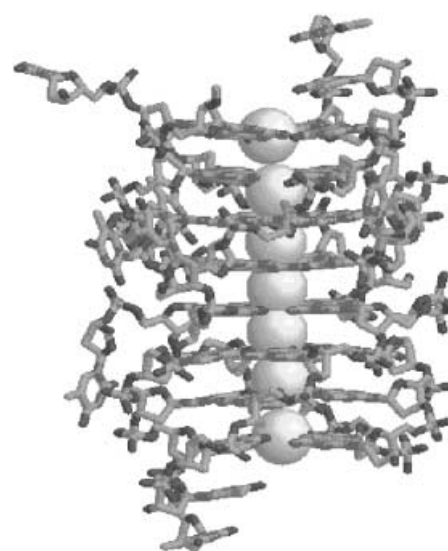


Figure 10. Crystal structure of the Na^+ form of the parallel-stranded G-quadruplex $[d(TGGGGT)]_4$.^[84] The seven colinear Na^+ ions give the impression of an ion channel. PDB code for this structure: 352D.

quartets, stack with the 5' ends in the same orientation to give a column of eight G-quartets. The four-stranded DNA, with seven colinear Na^+ ions, looks much like an ion channel, and very much resembles the lipophilic G-quadruplex $[1]_{16} \cdot 3K^+ \cdot Cs^+ \cdot 4pic^-$ shown in Figures 3 and 4.^[29] The Na^+ ions in this parallel DNA tetraplex are either sandwiched between G-quartets or coplanar with a G-quartet.

Electrostatic repulsion between such tightly packed cations must be diminished by coordination to the oxygen atoms of the G-quartet. *Ab initio* calculations show charge transfer from the G-quartet oxygen atoms to the bound cation.^[85] Other calculations conclude that the carbonyl–cation enthalpies provide more stabilization than either hydrogen bonding or stacking interactions.^[86] Furthermore, molecular dynamics simulations indicate that G-quadruplex DNA is stable only when Na^+ ions are bound;^[87] removal of the cations from the channel results in structural collapse.

An oligonucleotide with two G-rich regions can fold into a hairpin held together by G–G Hoogsteen

pairs (Figure 9b,c). Dimerization of a hairpin structure then provides a bimolecular G-quadruplex with two loops. These loops can orient in two ways to give different folds: “edge-wise” loops connect adjacent antiparallel chains, whereas “diagonal” loops cross over the G-quadruplex to connect antiparallel chains. In the early 1990s studies on the telomere repeat unit $d(G_4T_4G_4)$ of *Oxytricha* suggested that this sequence adopted different structures with different cations.^[88] The NMR solution structure with Na^+ ions showed a bimolecular G-quadruplex with diagonal loops,^[89,90] whereas a crystal structure of the K^+ form of $[d(G_4T_4G_4)]_2$ had “edgewise” loops.^[91] The significance of these differences has been diminished by more recent crystal structures of the K^+ form of $[d(G_4T_4G_4)]_2$ that, like the NMR structures,^[92] also show a bimolecular quadruplex with diagonal loops.^[59,60] The secondary structure of the G-quadruplex can be used to template the synthesis of other unusual DNA topologies. For example, Chan et al. took advantage of the shape of bimolecular G-quadruplexes to carry out a phosphodiester ligation on a single stranded DNA to provide circular oligonucleotides (Figure 11).^[93]

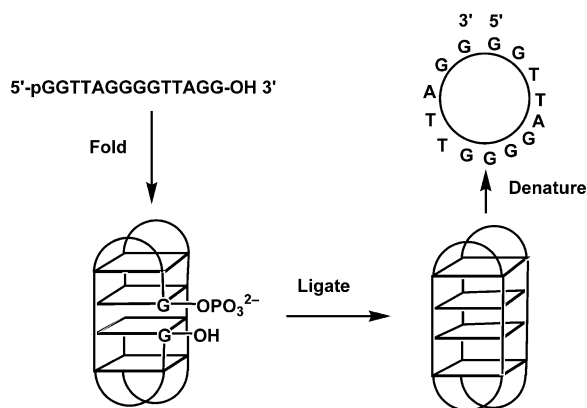


Figure 11. Synthesis of circular DNA oligonucleotides using a bimolecular G-quadruplex as template.^[93] A cation-promoted folding of the DNA single strand is followed by chemical ligation and denaturation to give the circular DNA.

Unimolecular G-quadruplexes, with three loops, form from a single strand (Figure 9d).^[94] Usually the loops constrain the G-quadruplex chains to be all antiparallel. Crystal structures of oligonucleotides containing the human telomeric sequence $d(TTAGGG)$ in their K^+ -bound form add another loop geometry to the G-quadruplex family.^[95] The 22-mer $d(AGGG(TTAGGG)_3)$ forms an intramolecular G-quadruplex in which all three TTA loops undergo a “double-chain-reversal” to give a unimolecular G-quadruplex with four parallel chains. These TTA loops extend far from the G-quartet core, thus providing potential sites for protein recognition (Figure 12). Furthermore, this structure, with its extended loops and exposed G-quadruplex core, suggests how different TTAGGG repeats might pack within the chromosome.^[96]

G-quadruplexes are cation-dependent and are stabilized by alkali and alkaline-earth cations. K^+ and Na^+ ions are

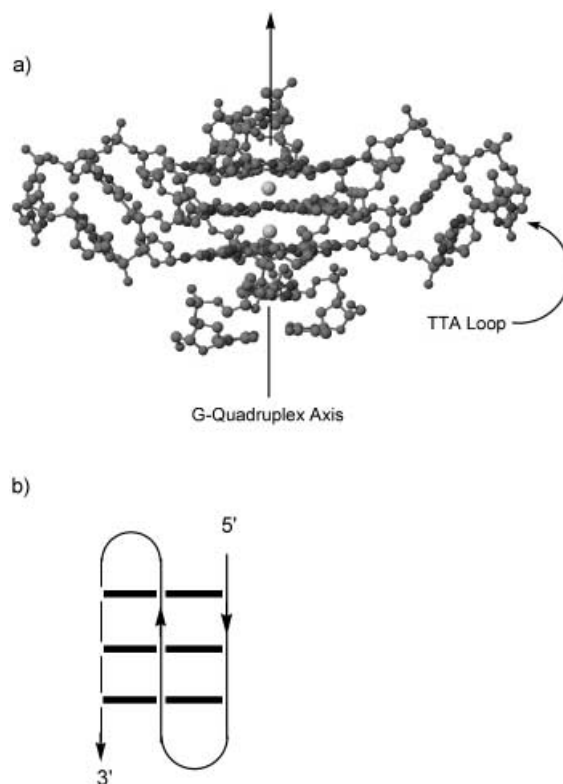


Figure 12. Crystal structure of an oligonucleotide $d(AGGG(TTAGGG)_3)$ containing the human telomeric sequence.^[95] a) Side view of the G-quadruplex showing the extended TTA loops. b) Schematic representation showing the “double-chain-reversal” that occurs within the TTA loops. PDB code for this structure: 1K8P.

prevalent in the cell, and thus these cations have received most attention.^[97] In general, DNA/RNA G-quadruplexes bind K^+ over Na^+ ions. This selectivity is often attributed to the better fit of K^+ ions into a G_8 -octamer cage. Ross and Hardin, however, concluded that the “optimal fit” proposal did not fully explain the K^+/Na^+ selectivity of the G_8 -octamer and suggested electronic factors must be important.^[98] Later, Hud et al. provided evidence that the hydration energy of the cations was the major determinant of K^+/Na^+ selectivity.^[99] While both cations fit between G-quartets, it is easier to dehydrate K^+ ions. Calculations support this ion dehydration argument.^[100]

In addition to oligonucleotides, polymers with modified backbones also form G-quadruplexes. Peptide nucleic acids (PNA) are nucleic acid mimics where the charged backbone is replaced by uncharged *N*-(2-aminoethyl)glycine linkages.^[101] Unlike DNA, hybridization and self-association of neutral PNA is not destabilized by electrostatic repulsion between the strands. Complementary C-rich PNA can invade and unfold intramolecular G-quadruplexes.^[102–104] Recently, Armitage and co-workers reported that G-rich PNA can also participate directly in four-stranded structures.^[105] They showed by using CD and fluorescence resonance energy transfer (FRET) that 1:1 mixtures of homologous DNA and PNA sequences assemble to form a stable hybrid $PNA_2:DNA_2$ G-quadruplex (Figure 13). Their findings

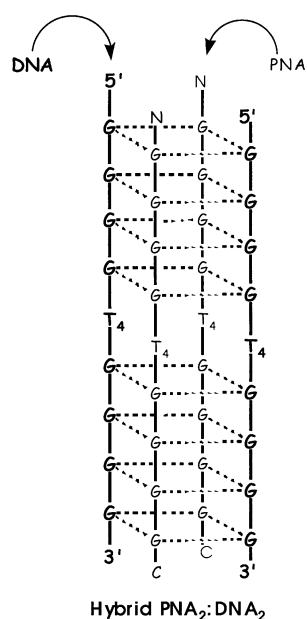
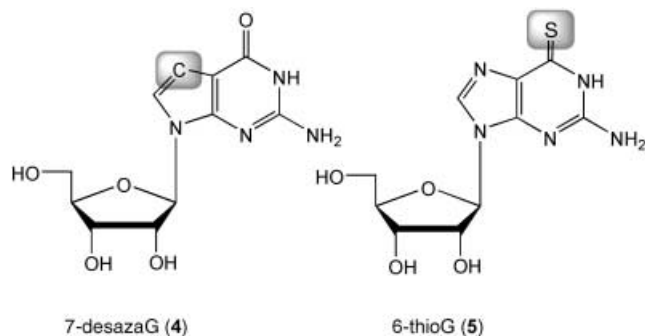


Figure 13. Proposed structure for a PNA₂-DNA₂ hybrid G-quadruplex, with the DNA (bold) and PNA strands diagonally opposed to each other.^[105]

expand the scope of PNA in molecular recognition processes and also suggest that PNA deserves consideration as a potential therapeutic toward targeting G-quadruplexes.

Sometimes G-rich DNA forms unwanted secondary structures. For example, DNA sequencing and polymerase chain reaction (PCR) amplification of G-rich sequences is often challenging because of extensive self-association. Modified nucleobases that can form G-C Watson-Crick base pairs, but that can't self-associate, have been used to circumvent such problems. Mizusawa et al. showed that 7-deazaG (**4**) enables accurate nucleotide sequencing of G-rich



DNA.^[106] Later, Seela and Mersmann demonstrated that disaggregation of the RNA G-quadruplex [r(UGGGU)]₄ could be effected by sequentially replacing G with 7-deazaG (**4**),^[107,108] which indicates that removal of the N(7) atom of guanosine gives a system that does not form stable G-quadruplexes. A decreased propensity to form G-quadruplexes was also observed for oligonucleotides containing 6-

thioG (**5**).^[109] The molecular basis for this effect may be a consequence of the significantly larger van der Waals radius of the sulfur atom, as well as its poorer hydrogen-bonding and cation binding abilities. Molecular dynamics of DNA containing 6-thioG (**5**) supported the hypothesis that a sulfur atom at C6 is just too big to form a G-quartet.^[110]

3.2. G-Quartets Stabilize Stacking of Nucleobase Triads and Tetrads

As shown in Figure 14 and Table 2, there are different ways in which molecules can interact with G-quadruplexes: by face recognition, edge recognition, loop recognition, or by simultaneous binding to the surface of the G-quartet and an adjacent loop or groove.

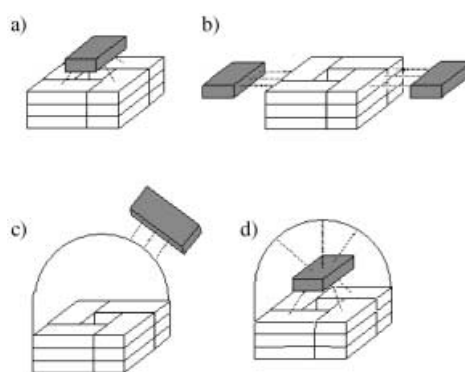


Figure 14. Schematic representation showing some of the ways that other molecules recognize a bimolecular G-quadruplex. a) "Face" recognition is achieved by stacking interactions, either on the terminal G-quartet or by intercalating between G-quartet layers; b) "edge" recognition occurs with the exposed N3, N2-H, and C8-H atoms on the G-quartet; c) binding of molecules to single-stranded loops connected to the G-quadruplex core; d) simultaneous "end" stacking and interaction of molecules with adjacent loop or groove regions.

Table 2: Molecular recognition of G-quadruplexes.

face recognition through stacking:

- nucleobase triads and tetrads
- aromatic molecules/telomerase inhibitors

edge recognition through hydrogen bonding:

- hydrogen bonding of other nucleobases
- protein recognition: edge interactions with amino acids
- water-nucleobase interactions

loop recognition through hydrogen bonding and electrostatic interactions:

- protein interactions: electrostatic phosphate-ammonium interactions
- small molecules and water

groove recognition through hydrogen bonding and electrostatic interactions

- water interactions with the hydration spine
- side-chain interactions with stacked aromatic molecules

Within the context of nucleic acids, there are two major ways for adjacent nucleobases to recognize a G-quartet: either by stacking on its surface or by hydrogen bonding to an exposed edge. Furthermore, neighboring loops in bimolecular and unimolecular G-quadruplexes are also molecular recognition sites, particularly for proteins and small molecules.^[59] One emerging theme from biophysical studies is that the G-quartet provides a platform on which to stabilize other hydrogen-bonded motifs.^[9] Cheong and Moore first showed this with $[\text{r}(\text{UG}_4\text{U})_4]$.^[111] From the NMR spectrum they deduced a structure that featured a hydrogen-bonded U_4 -quartet stacked above the G-quadruplex. They suggested that the U_4 -quartet forms because it was "... in the special context provided at the end of a tetra G stack, not because it is an intrinsically stable structure".^[111] Patel and co-workers have used the G-quartet to template formation of base triplets and quartets,^[112–116] and emphasize that stacking interactions stabilize these hydrogen-bonded arrangements. One example that illustrates the concept is the 12-mer $\text{d}(\text{A}_2\text{G}_2\text{T}_4\text{A}_2\text{G}_2)$, which dimerizes in the presence of Na^+ ions to give a diamond structure, with symmetry-related G-quartets at its core.^[114] The diamond features progressive stacking of a G-quartet, a T-[A-A] base triplet, a mismatched dimer, and a single base (Figure 15). These studies highlight the ability of the G-quartet to expand the structural diversity of DNA. Unusual structures, such as this diamond, should be prime candidates for binding small molecules and proteins.

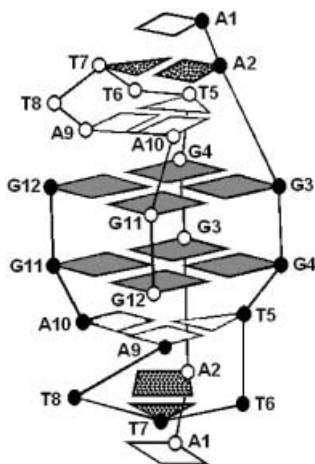


Figure 15. NMR spectroscopically derived "diamond" structure formed upon bimolecular association of the oligonucleotide $\text{d}(\text{A}_2\text{G}_2\text{T}_4\text{A}_2\text{G}_2)$ in the presence of Na^+ ions. The G-quartet core acts as a platform on which to stabilize adjacent T-[A-A] base triads.^[114] The T residues are depicted as triangles and the G/A residues as rectangles.

Base tetrads, such as U_4 , T_4 , and A_4 , can cap G-quadruplexes.^[111,117,118] Stable mixed tetrads with more than one nucleobase type also form above the G-quartet. Patel et al. showed that $\text{d}(\text{GCGGT}_3\text{GCGG})$ dimerizes in solution to give a quadruplex with G:C:G:C tetrads stacked above the G-quartets.^[119] These mixed G:C:G:C tetrads are dimers of Watson–Crick G:C base pairs which switch between distinct geometries (Figure 16) depending on the ionic conditions.

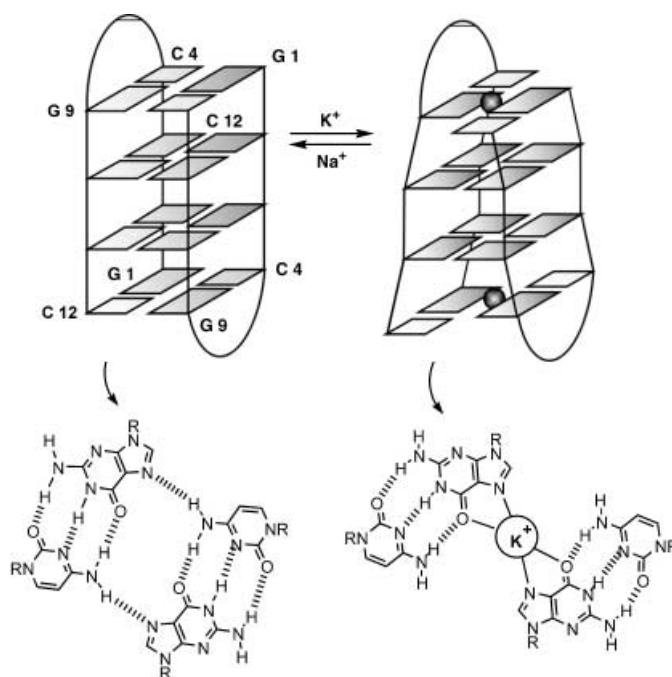


Figure 16. A cation-templated conformational change for the bimolecular G-quadruplex formed by $\text{d}(\text{GGGCT}_4\text{GGGC})$. A G:C:G:C tetrad with directly opposed G-C base pairs is stacked above a G-quartet in Na^+ solution. These four bases adopt a "slipped" alignment of the G-C pairs upon complexation of K^+ by the N7 and O6 atoms of the guanine.^[120–122] The organization of the G:C:G:C tetrad is shown below the schematic diagrams.

NMR studies of $\text{d}(\text{GGGCT}_4\text{GGGC})$ in Na^+ solution showed a G:C:G:C tetrad with directly opposed G-C pairs.^[119] Transition to a slipped alignment of G-C pairs occurred upon addition of K^+ ions, presumably as a result of cation chelation by the N7 and O6 atoms of the guanine.^[120] Both ab initio and molecular dynamics calculations reproduced this K^+ -induced transformation between the "closed" and "sheared" forms of the G:C:G:C tetrad.^[121,122] Both G:C:G:C geometries show extensive overlap of their aromatic surfaces onto the flanking G-quartet. Extensive base stacking along the length of this G-quadruplex undoubtedly stabilizes its fold. These studies show that DNA quadruplexes don't need to be entirely composed of G-quartets. Furthermore, the propensity of G-rich sequences to template formation of base tetrads and triads also greatly increases the structural variability of DNA.

3.3. Molecular Recognition of the G-Quartet Edges

Hydrogen-bond donor (N2-H and C8-H) and acceptor (N3) atoms on the exposed edges of the G-quartet are also molecular recognition sites (see Figure 1 b). These sites, which are located along the floor of a groove running the length of the G-quadruplex, can bind water, ions, amino acid side chains, and other nucleobases. For example, some G-quartets form "sheared" G-A hydrogen bonds with a local adenine residue. NMR studies have identified hexad and heptad

motifs, where two or three adenine nucleobases hydrogen bond to a single G-quartet.^[123–126]

Uesugi and co-workers found in an NMR study of r(GGAGGUUUUGGAGG) that one RNA molecule folds into a quadruplex containing a G:G:G:G tetrad and a G:G:(A):G:G:(A) hexad.^[125] As depicted in Figure 17, expansion

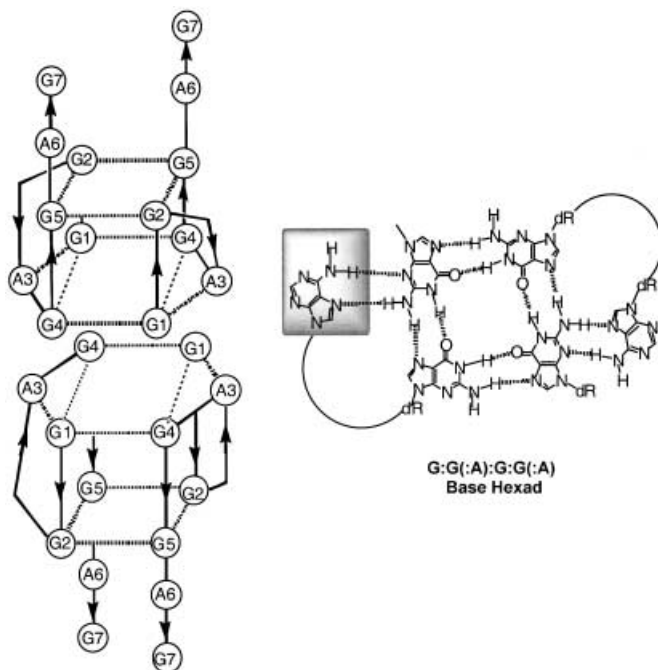


Figure 17. Edge recognition of a G-quartet through formation of hydrogen bonds between nearby adenine units to the exposed face of the quartet. One molecule of r(GGAGGUUUUGGAGG) folds into a G-quadruplex containing a G:G:G:G tetrad and a G:G:(A):G:G:(A) hexad. Two such RNA molecules dimerize in solution using this hexad–hexad interface.^[125]

sion of the lower G-quartet into a hydrogen-bonded hexad also increases the surface area available for stacking interactions, and two r(GGAGGUUUUGGAGG) molecules dimerize in solution using this hexad–hexad interface. The extensive base-stacking and hydrogen-bonding interactions contribute to the high thermodynamic stability of this particular RNA quadruplex. A crystal structure of a Sr²⁺-bound RNA showed the ultimate in base expansion: an octad, with one G:U pair formed for every member of a G-quartet.^[126] These studies indicate that recognition of a G-quartet edge may be an important theme in nucleic acid folding.

3.4. Molecular Recognition of G-Quadruplexes by Water

Crystal structures provide a wealth of information about the molecular recognition of G-quadruplexes.^[59,60,84] In particular, these structures indicate that water helps maintain the structural integrity of DNA. A G-quadruplex has four grooves on its surface and the floors of these grooves consist

of the C8-H, N2-H, and N3 atoms. Above these floors, the grooves are lined on both sides by the oxygen atoms of phosphate and sugar groups. All these atoms, on the floor and along the edges, can form hydrogen bonds with water molecules and there are, indeed, many different combinations of cross-connections possible.^[59,60,84] Horvath and Schultz observed that hydrogen bonds involving C8-H are a key feature of G-quartet hydration in the *Oxytricha* structure, with 7 of 25 bound water molecules forming hydrogen bonds to the aromatic C8-H proton.^[59] Water molecules make extensive hydrogen-bonding interactions with the edges of the guanine units and with the sugar-phosphate backbone to provide well-ordered hydration spines in the grooves of the G-quadruplex. An example of a hydration spine, from the [d(TG₄T)]₄ structure, is shown in Figure 18.^[84]

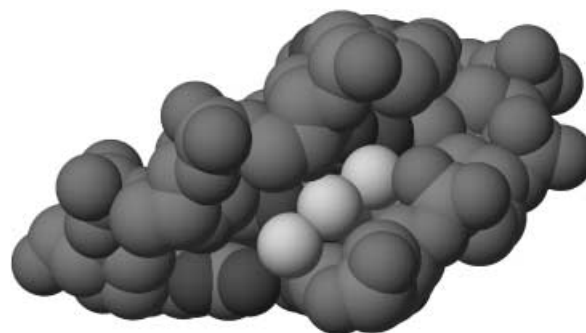


Figure 18. View of a hydration spine filling one of the grooves in the Na⁺ form of the G-quadruplex [d(TGGGGT)]₄.^[84] The light-colored atoms are the water oxygen atoms. PDB file for this structure: 352D.

Neidle and co-workers detailed the hydration pattern of this bimolecular G-quadruplex in their study of *Oxytricha* DNA.^[60] Again, all the grooves had water clusters that bridged adjacent DNA strands. In addition, the G-quadruplex loops were also extensively hydrated, and water molecules connected the loops to the terminal K⁺ ion and to the hydration spines running along the grooves. Water-mediated bridges between domains surely help to stabilize the loop conformations.

3.5. Molecular Recognition of G-Quadruplexes by Proteins

DNA and RNA oligonucleotides called aptamers have been selected by in vitro selection to bind molecular targets.^[127] These targets are often proteins. One well-known aptamer is the thrombin binding aptamer (TBA), a DNA 15 mer that inhibits clotting.^[128] Crystallographic^[129,130] and NMR^[131–134] spectroscopic studies have shown that TBA forms a unimolecular G-quadruplex with two G-quartets and three loops. The structure and biological activity of TBA are K⁺-dependent. NMR spectroscopic, calorimetric, and ESI-MS studies have shown, however, that TBA is even more stable with divalent Pb²⁺, Sr²⁺, and Ba²⁺ ions.^[135–137] Related DNA aptamers are inhibitors of human HIV integrase with IC₅₀ values in the nm region^[138,139] and adopt a unimolecular

G-quadruplex similar to TBA.^[140,141] An intermolecular G-quadruplex aptamer that bound the V3 loop of HIV reverse transcriptase was formed by a 17-mer phosphorothioate oligonucleotide.^[142] Like many G-quadruplexes, this phosphorothioate aptamer was extremely stable and had a dissociation half-life of 60 days.^[143] Thus, it remained active even at μM concentrations. Many other protein-binding aptamers have been proposed to form G-quadruplexes as part of their bioactive structure.^[144–148]

Although many proteins, including aptamers, oncogenic factors,^[149a] antibodies,^[150] and telomeric proteins,^[12–14] all bind G-quadruplexes, there are few molecular details known about quadruplex–protein interactions.^[12] The crystal structure of TBA bound to thrombin showed some ion pairs between the phosphate groups in the loops of the aptamer and Lys and Arg side chains.^[129,130] A crystal structure of the telomeric protein of *Oxytricha nova* complexed to $d[\text{G}_4\text{T}_4\text{G}_4]_4$ is particularly valuable.^[59] As in the DNA solution structures, the bimolecular quadruplex in this complex has diagonal loops. Most of the DNA–protein contacts take place with these loops, rather than with the quadruplex core.

The major DNA–protein interactions include: 1) electrostatic interactions, wherein the surface formed by three symmetry-related proteins provides a deep, electropositive cavity to hold the folded DNA; 2) van der Waals interactions, such as where the aromatic rings of Tyr 142 and Phe 141 pack against the G-4 sugar; 3) water mediated hydrogen bonds, wherein one of the T_4 loops forms an extensive network of water-mediated hydrogen bonds with the protein; 4) nucleobase–peptide packing interactions, wherein the T6 residue of the other loop contacts the protein using both van der Waals and H-bond interactions. This T6 nucleobase packs between a Leu side chain and the polarized Asp437–Gly438 peptide bond, while simultaneously hydrogen bonding with atoms of the protein side chain and main chain.

In both cases where crystal structures are available, for the *Oxytricha* and for the thrombin systems,^[59,129,130] there are few direct interactions between the protein and the G-quadruplex core. Instead, the G-quadruplex seems to function as a scaffold on which loops are displayed for molecular recognition. Certainly, the crystal structure of the unimolecular G-quadruplex formed from the human telomeric sequence $d(\text{TTAGGG})$ suggests that the extended loops provide ideal protein-binding sites.^[95] Wen and Gray have also proposed that g5p—a protein that binds single-stranded DNA—may prefer to bind constrained loops, as opposed to random coils, to reduce its entropic binding cost. This might be best accomplished by having a G-quadruplex core constrain the conformation and dynamics of nearby loops.^[147] In time, as more structural information becomes available, we should learn if a preference to bind loops is general for G-quadruplex–protein interactions.^[149]

3.6. G-Quadruplex Aptamers: Recognition by Small Molecules

Small molecules can also generate DNA and RNA aptamers, and G-quartets often appear as part of the aptamer structure.^[151,152] Porphyrins and G-quartets have similar sur-

face areas, and many biophysical and biochemical studies have demonstrated stacking interactions between porphyrins and G-quadruplexes.^[79,153–158] Sen and co-workers as well as other research groups have described some oligonucleotide aptamers that bind to porphyrins.^[159–164] On the basis of optical spectroscopy and chemical protection evidence it was proposed that these aptamers folded into G-quadruplexes in the presence of the porphyrin. These studies went beyond demonstrating ligand–receptor binding: some aptamers, selected with transition-state analogue *N*-methylmesoporphyrin (NMM), catalyzed the Cu^{2+} and Zn^{2+} metalation of porphyrins (Figure 19).^[161] This catalytic DNA, which

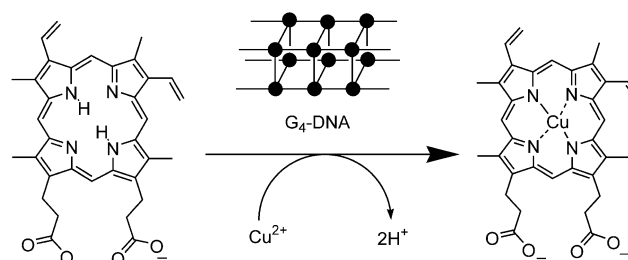


Figure 19. Porphyrin metalation catalyzed by a DNA G-quadruplex.^[161]

requires K^+ ions for activity, may bind the porphyrins either by external stacking or by intercalation between G-quartets. Li and Sen concluded that the DNA chelates used substrate binding energy to distort the planar conformation of the porphyrin, thus making the porphyrin more basic and easier to metalate.^[163] They suggested that the G-quartet is sufficiently rigid to enable such a substrate distortion.^[164]

Sen and co-workers also identified DNA aptamers that catalyze another reaction.^[165,166] Some DNA–hemin complexes had enhanced peroxidase activity relative to the heme cofactor alone. They concluded that the folded DNA activates the bound heme.^[165] A G-quadruplex with a heme intercalated between G-quartets was proposed to explain the enhanced peroxidase activity of the aptamers.^[166] These studies underscore the potential of DNA to function as a catalyst, in addition to its information storage role.

By using *in vitro* selection Isalen et al. discovered Zn^{2+} finger peptides that bind G-quadruplexes.^[167] High affinity peptides ($K_a = 25 \text{ nM}$) containing three helices bound the human telomeric sequence $d(\text{GGTTAG})$. These Zn^{2+} fingers were G-quadruplex-specific; duplex DNA with the same sequence was not bound. The Zn^{2+} fingers contained high amounts of certain amino acids, and the authors speculated that Glu/Asp side chains might form hydrogen bond with the N2 amino groups of the guanosine while His groups could stack on the G-quartet.

3.7. Telomerase Inhibitors: Small Molecules that Stabilize DNA G-Quadruplexes

The telomere is a protective nucleoprotein complex located at the ends of chromosomes.^[168–171] In humans,

telomeric DNA is a 5000–15 000 base section of 5'-TTAGGG-3' repeats bound by an array of proteins. Since 50–500 TTAGGG units are lost with each replication, the telomere is slowly whittled away and the cell dies. Tumor cells evade this fate by expressing telomerase, a reverse transcriptase with a RNA template for telomere extension.^[172–174] Telomeres are proposed to be involved in cancer, based in part on the finding that *in vitro* telomerase induction can transform healthy cells into malignant ones.^[175] Furthermore, telomerase inhibition can kill cancer cells, "... validating human telomerase ... as an important target for anti-neoplastic therapies."^[176]

One approach to inhibit telomerase is to block the enzyme–substrate interaction. Whereas single-stranded DNA is a telomerase substrate, G-quadruplex DNA is not. The single stranded overhang of 5'-TTAGGG-3' repeats in the human telomere has been proposed to fold into an intramolecular G-quadruplex, and ionic conditions that stabilize G-quadruplexes inhibit telomerase. Cech and co-workers reported that the telomerase activity of *Oxytricha nova* dropped significantly in the presence of K⁺ ions: "... (f)olding of telomeric DNA into G-quartet structures seems to influence the extent of telomere elongation *in vitro* and might therefore act as a negative regulator of elongation *in vivo*."^[177]

Telomerase inhibition depends on shifting the equilibrium between the single-strand and quadruplex towards the folded form. One strategy is to stabilize the G-quadruplex form by binding high-affinity small molecules. A number of reviews describe well how drug design and screening efforts have identified compounds that bind G-quadruplexes and inhibit telomerase.^[178–183] As shown by the structural formulas 6–15, most telomerase inhibitors are aromatic compounds with electron-deficient rings suited for stacking with the electron-rich G-quartet. Ethidium bromide (6), a well-known duplex

intercalator, binds DNA quadruplexes,^[184] but its surface area doesn't quite cover a G-quartet, and it was reasoned that larger aromatic compounds would be better inhibitors.

The first report of a small molecule, anthraquinone derivative 7, that binds G-quadruplex DNA and inhibits telomerase came in 1997.^[185] Since then, hundreds of telomerase inhibitors have appeared.^[178–183] There is debate about how the aromatic inhibitors interact with G-quadruplex DNA. Some suggest that they intercalate between G-quartets,^[186] while others argue that the compounds end-stack on terminal G-quartets.^[155] The few known quadruplex–ligand structures, obtained from NMR spectroscopic,^[187,188] fiber diffraction,^[189] and single-crystal X-ray crystallographic analysis,^[190,191] indicate that telomerase inhibitors end-stack rather than intercalate (Figure 20). In their recent X-ray study of

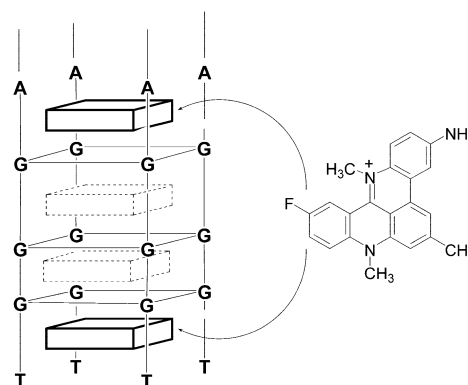
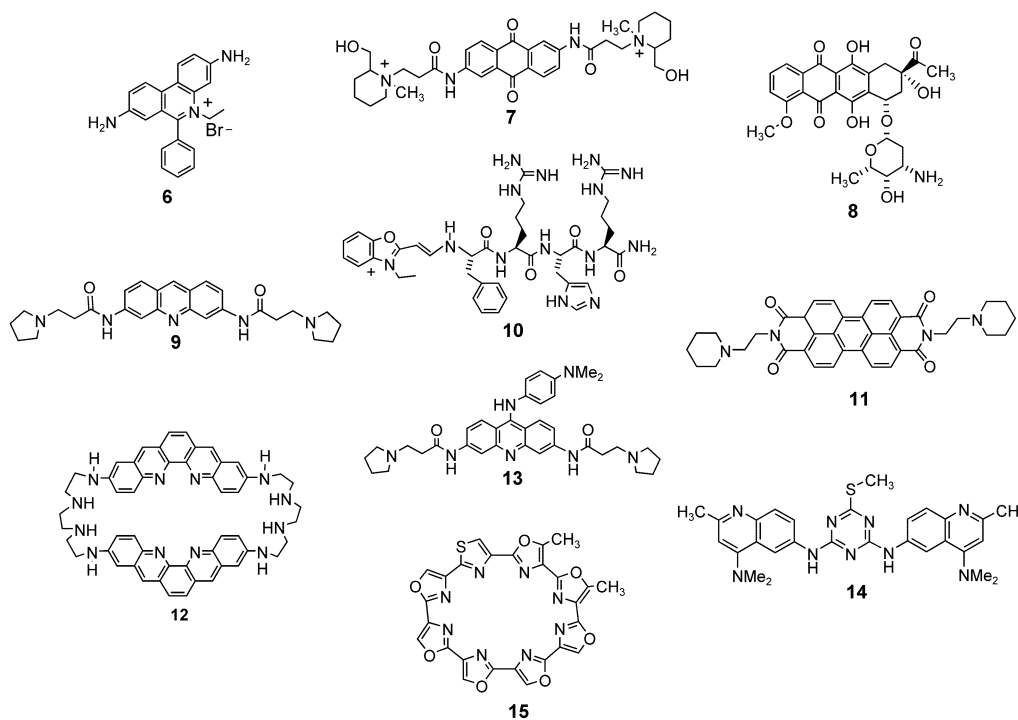


Figure 20. Interaction of the aromatic inhibitor with G-quadruplex DNA: NMR data show the drug stacking on ends of the G-quadruplex (bold), as opposed to intercalation between the G-quartet layers (dotted).^[188]



interactions between G-quadruplex and daunomycin (**8**) Clark et al. noted that intercalation of drugs between G-quartet layers should be energetically more costly than end stacking, as intercalation requires unstacking of the tetrad and unwinding of the helix.^[191] In addition, end stacking of aromatic drugs, as opposed to intercalation, would allow stabilizing cations to remain coordinated within the G-quadruplex core. In another crystal structure of a G-quadruplex–drug complex, the drug **9** not only end-stacks on the terminal G-quartet, it also simultaneously binds the adjacent loop with its side chains to secure its position.^[190]

Structure-based design and activity-based screening have both identified telomerase inhibitors. For example, Shafer and co-workers used computer modeling to screen “virtual” libraries for compounds that might interact with G-quadruplexes. These efforts identified a carbocyanine dye that binds bimolecular G-quadruplexes.^[192] Recently, a carbocyanine–peptide conjugate **10**, identified by combinatorial selection, was shown to bind G-quadruplex DNA with high affinity and quadruplex/duplex selectivity.^[193]

In another modeling approach, Fedorov, Hurley, and co-workers designed and synthesized the quadruplex interactive compound PIPER (**11**), which was a potent telomerase inhibitor.^[187] The slow NMR exchange between DNA-bound and free PIPER (**11**), along with ligand–DNA NOE interactions, allowed determination of the drug's location when bound to G-quadruplex DNA. The conclusion was that PIPER (**11**) end-stacked over a terminal G-quartet. Significantly, this perylene derivative is also a molecular chaperone, as it accelerated the formation rate of a bimolecular G-quadruplex by 100-fold.^[194] Salazar and co-workers took advantage of this affinity in designing a PIPER conjugate, perylene-[Fe^{II}(edta)] for cleavage of G-quadruplex DNA.^[195] They used NMR spectroscopy to establish that the Fe-free perylene stacked on the terminal G-quartet. They then demonstrated that perylene-[Fe^{II}(edta)] selectively cleaved G-quadruplex DNA without damaging duplex DNA. Compounds that selectively destroy G-quadruplex DNA could be useful structural probes.

Recent work suggests that G-quadruplexes, themselves self-assembled structures, also interact with aggregated ligands. These findings are reasonable, when the extensive surface area provided by a planar G-quartet is considered. Although the structural basis is unclear, Kerwin and co-workers showed that the binding selectivity of the tetraplex is greatly enhanced when PIPER (**11**) is aggregated.^[196,197]

A crystal structure illustrates this remarkable self-assembly feature of G-quadruplex:drug interactions. Clark et al. showed that daunomycin (**8**) self-assembles into a noncovalent trimer [**8**]₃ when interacting with four-stranded [d(TG₄T)]₄ (Figure 21). The daunomycin trimers are held together by van der Waals interactions. In this crystal, two daunomycin trimers stack between the 5'-G-quartets that make up the G-quadruplex.^[191] There are also extensive stacking interactions between the terminal G-quartet and the daunomycin trimer. In addition, sugars on the three daunomycin molecules extend into the grooves where they hydrogen bond with the G-quadruplex core.

Competition dialysis experiments recently showed that the dimeric macrocycle BOQ (**12**), unlike its monomeric analogue, selectively binds G-quadruplex DNA.^[198] Compound **12** is also a potent telomerase inhibitor. The selectivity of the dimeric macrocycle, relative to the monomeric form, is attributed to its enhanced stacking and hydrophobic interactions with G-quadruplex DNA.

Read et al. have shown how structure-based design can be used to obtain improved telomerase inhibitors.^[199] Molecular modeling studies suggested that addition of a properly positioned third side chain to an existing acridine drug **9** (to give **13**) would increase the affinity of the G-quadruplex. They reasoned that additional contacts between the side chains and grooves would further stabilize the folded DNA, and thus lead to more telomerase inhibition. A comparison of the properties of acridines **9** and **13** showed that attachment of this third side chain improved the drug's affinity to bind with the G-quadruplex, its quadruplex–duplex selectivity, and its telomerase inhibitory activity.

Promising telomerase inhibitors have also come from combinatorial screening strategies. The research groups of Mergny and Hélène used a FRET-based assay to identify triazine compounds such as **14** that induce a DNA oligonucleotide to fold into a G-quadruplex.^[200,201] Some of these triazines were inhibitors of human telomerase at nanomolar concentrations. Such combinatorial screens can lead to active compounds that might not have been considered in structure-based design.

Finally, the most potent and selective telomerase inhibitor, telomestatin (**15**), is a natural product isolated from *Streptomyces anulatus* during an activity screen.^[202] NMR and modeling studies indicate that telomestatin stacks on a G-quartet, which again suggests that telomerase inhibition derives from an ability to stabilize the G-quadruplex form of telomeric DNA.^[203]

3.8. Summary of Interactions Important for G-Quadruplex Structure and Molecular Recognition

As for most supramolecular systems, different noncovalent interactions work in concert to provide a G-quadruplex. Both experiment and calculations show that cation–dipole interactions are essential for the hydrogen bonding and base stacking that is characteristic of G-quadruplex structures. Cation binding presumably reduces the repulsion of the four central oxygen atoms of the hydrogen-bonded quartet, enhances hydrogen-bond strength, and stabilizes G-quartet stacking.

Stacking interactions are also clearly important in controlling the structure and molecular recognition of G-quadruplex DNA. Base stacking is driven by electrostatic and dispersive (van der Waals) interactions.^[204,205] The G-quartet provides an extended surface for interactions with aromatic compounds, be they other nucleobase assemblies or small molecule ligands. While there is debate over the relative importance of the forces that drive aromatic-stacking interactions,^[204] both calculations and experiment suggest that the

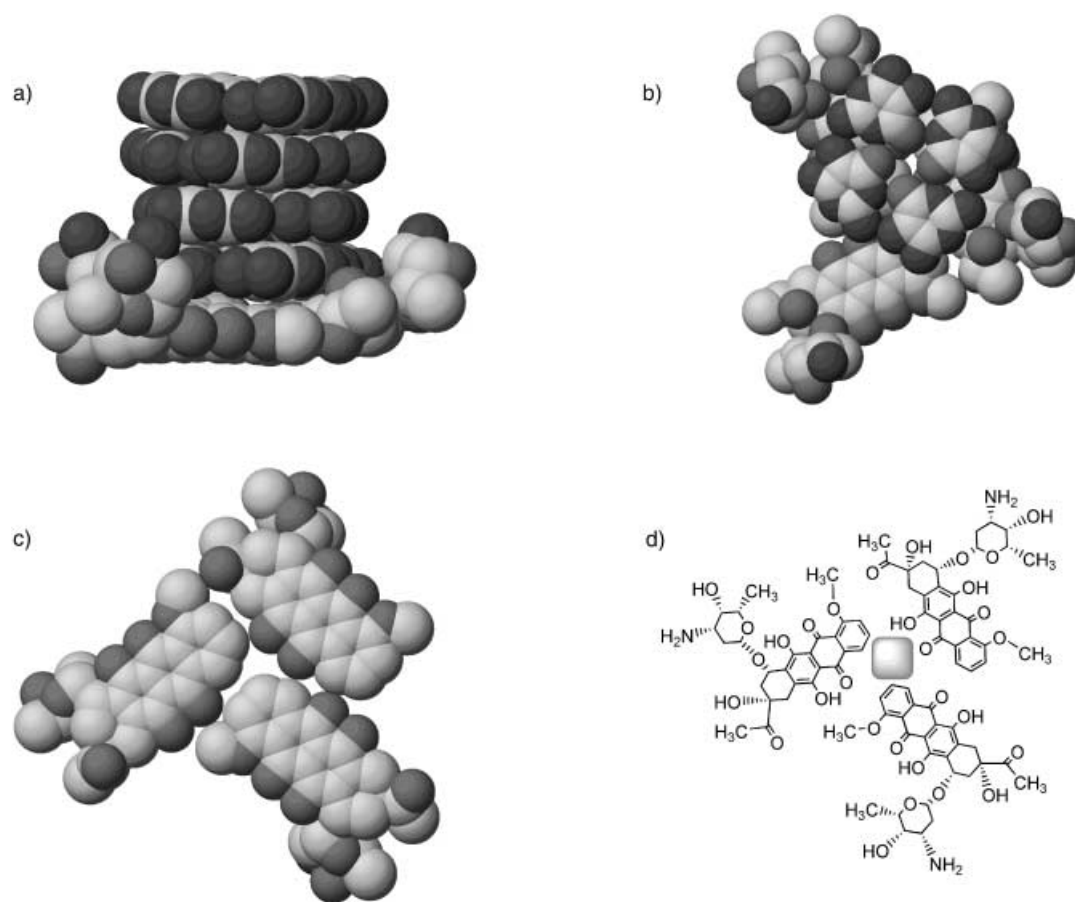


Figure 21. Depictions of the crystal structure showing a self-assembled daunomycin trimer [8]₃ bound to the four-stranded G-quadruplex [d(TG₄T)]₄.^[191] a) The G-quartet core stacked on top of [8]₃; b) a top view of the terminal G-quartet stacked on trimer [8]₃; c) bottom view of the daunomycin trimer held together by van der Waals interactions; d) the structural formula of the daunomycin trimer [8]₃. PDB code for this crystal structure: 1O0K.

nonclassical hydrophobic effect can explain such stacking interactions.^[205] For example, Spöner and co-workers calculated that van der Waals interactions between two G-quartets are strongly attractive, with $\Delta H = -49 \text{ kcal mol}^{-1}$, while the electrostatic component was repulsive, with $\Delta H = +28 \text{ kcal mol}^{-1}$, which gives an overall stacking energy of $\Delta H = -21 \text{ kcal mol}^{-1}$, even in the absence of the templating cation.^[87] This enthalpy for quartet stacking ($\Delta H = -21 \text{ kcal mol}^{-1}$) is greater than the enthalpies of $\Delta H = -10$ to $-15 \text{ kcal mol}^{-1}$ calculated for stacking interactions within duplex DNA. Calorimetry measurements on G-quadruplex–ligand interactions also show the hallmarks of the “non-classical” hydrophobic effect, with $\Delta H \ll 0$ and $T\Delta S \ll 0$.^[186] This effect arises from the enthalpic gain of stacking large aromatic surfaces containing polarizable atoms.^[205] There is also an enthalpic gain when weakly bound water is released from these aromatic surfaces to the bulk.

Finally, hydration spines within the grooves and the water molecules that link the loops to the G-quartet core also help control G-quadruplex structure, dynamics, and function.

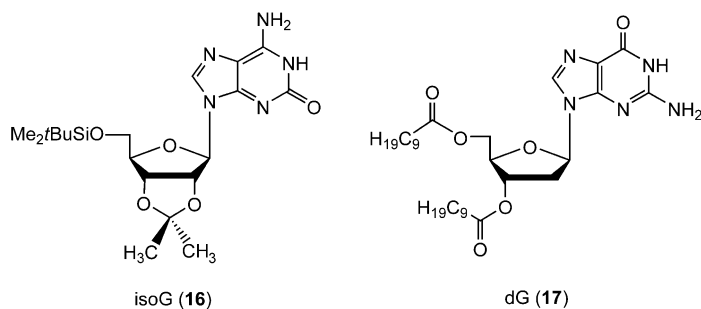
4. Lipophilic G-Quadruplexes: Structural Models for DNA, Self-Assembled Ionophores and Other Structural Motifs

In the 1990 review by Guschlbauer et al. it was stated that “(w)ater appears to be an indispensable solvent for the auto-association of guanosine ... organic solvents give rise to poorly organized aggregates”.^[1] At that time, the analogues and conditions needed to form G-quadruplexes in organic solvents had not yet been identified. Since then it has been found that lipophilic nucleosides do indeed form stable, well-ordered species in organic solvents. These lipophilic G nucleosides are used as models for DNA G-quadruplexes, as the basis for studying noncovalent interactions, and as the inspiration for synthetic molecular assemblies.

4.1. Early Intermediates in Guanosine Self-Assembly: Discrete G₈·K⁺ Octamers in Organic Solvents

While exploring base pairing in organic solvents, it was observed that lipophilic G (**1**) and its isoguanosine isomer

(isoG, **16**), both self-associate in the presence of cations.^[206,207] Concurrently, Gottarelli et al. reported the K⁺-templated self-assembly of 3',5'-didecanoyl-2'-dG (**17**) in CDCl₃.^[208] Depend-



ing on conditions, dG (**17**) can extract K⁺ picrate from water into CDCl₃ to give either a discrete octamer, [**17**]₈·K⁺, or a polymer, ([**17**]₄)_n·(K⁺)_m. Lateral self-organization of the stacked ([**17**]₄)_n·(K⁺)_m columns in hydrocarbon solvents gives hexagonally packed liquid crystals.^[209]

To better understand the transition from molecule to defined aggregate to ordered phase in organic solvents, we collaborated with Gottarelli et al. to determine the NMR structure of the octamer [**17**]₈·K⁺·I⁻ in CDCl₃, the first detectable intermediate in self-assembly and self-organization of dG (**17**).^[210] Remarkably, the NMR data indicated that this octamer was a single diastereomer. In one G-quartet, all dG units had a *syn*-glycosidic conformation, while the other tetramer had an “all-*anti*” conformation. NOE interactions indicated a “head-to-tail” orientation of the “all-*anti*” G-quartet stacked on the “all-*syn*” G-quartet, with a 30° twist about the central axis. Small angle neutron scattering and NMR data later showed that the ([**17**]₄)_n·(K⁺)_m polymer had the same G-quartet geometry and organization as did this discrete [**17**]₈·K⁺ octamer.^[211] This NMR study firmly established that discrete G-quartet structures exist in organic solvents. The study also showed that self-assembly of dG (**17**), followed by domain self-organization, was promising for building nanostructures (see Section 5). Structural characterization of discrete species such as [**17**]₈·K⁺·I⁻ provided a better understanding of self-assembly, and also generated ideas for new supramolecular designs.

4.2. Crystal Structures of Lipophilic G-Quadruplexes: Models for Locating Ions in DNA

Besides X-ray crystallography, there are few methods to directly locate cations in DNA G-quadruplexes. ¹⁵N NMR has provided insight into NH₄⁺ binding, and ²⁰⁵Tl NMR showed how a K⁺ surrogate stabilizes the [d(T₂G₄T₂)]₄ quadruplex.^[212,213] Although ²³Na NMR spectroscopy in solution provides dynamic information, it gives no structural insight.^[214] There is a need, therefore, for techniques that detect DNA-bound metals. The high-resolution crystal structures of lipophilic G-quadruplexes synthesized from G (**1**)^[29–33] have helped validate such new methods, namely

solid-state ²³Na and ³⁹K NMR spectroscopy and extended X-ray absorption fine structure (EXAFS).

Solid-state ²³Na NMR spectra were previously obtained for the DNA quadruplex [d(TG₄T)]₄.^[215] but assignment of the G-quartet-bound Na⁺ ion was compromised by atmospheric Na⁺ ions bound to the DNA phosphates. The lipophilic G-quadruplex [**1**]₁₆·3Na⁺/Cs⁺·4pic⁻ was an ideal model for circumventing such problems; it has three crystallographically distinct channel Na⁺ ions and no phosphate-bound Na⁺ ions to complicate the NMR analysis. Wu and co-workers used 2D ²³Na MQMAS NMR spectroscopy (MQMAS = multiple quantum magic angle spinning) to determine the isotropic chemical shifts and other parameters for the three Na⁺ sites within [**1**]₁₆·3Na⁺/Cs⁺·4pic⁻.^[33] The unambiguous detection of Na⁺ ions within the channel firmly established these lipophilic G-quadruplexes as excellent models for DNA G-quadruplexes.

Wu et al. also used solid-state ³⁹K NMR spectroscopy to identify K⁺ ions within the lipophilic G-quadruplex [**1**]₁₆·3K⁺/Cs⁺·4pic⁻.^[216] The quartet-bound K⁺ ions provided a distinctive ³⁹K NMR signal at δ = -45 ppm. Importantly, the ³⁹K chemical shift for this model then enabled the authors to distinguish different K⁺ ions in the 5'-GMP quadruplex; both the G-quartet-bound and phosphate-bound K⁺ ions were identified. This solid-state ³⁹K NMR study, the first spectroscopic detection of biologically relevant K⁺ ions, bodes well for studies of DNA-(G₄)_n·K⁺ quadruplexes.

Shafer and co-workers described the first use of EXAFS to characterize DNA–metal ion binding in solution.^[217] EXAFS provides coordination numbers and quantitative internuclear distances for the bound metal. The authors determined the location of the Pb²⁺ ions and the Pb–O distances for the thrombin binding aptamer d(G₂T₂G₂TGTG₂T₂G₂) (TBA). They had previously shown that this unimolecular G-quadruplex binds Pb²⁺ ions tightly,^[218] but it was unclear as to whether the Pb²⁺ ions were bound between the two G-quartets of the TBA, or coordinated to the terminal G-quartet and adjacent loop (Figure 22).^[219,220] The lipophilic G-quadruplex [**1**]₁₆·2Pb²⁺·4pic⁻ helped resolve this structural issue. The crystal structure had previously shown that an octacoordinate Pb²⁺ ion was sandwiched between two G-quartets.^[30] The EXAFS Pb–O distances for the model G-quadruplex

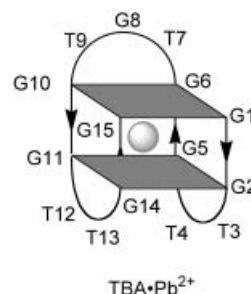


Figure 22. The thrombin binding aptamer (TBA) with a bound Pb²⁺ ion. Comparison of the EXAFS data for the lipophilic G-quadruplex [**1**]₁₆·2Pb²⁺ and for Pb²⁺·TBA helped identify the cation binding site.^[217]

$[1]_{16} \cdot 2\text{Pb}^{2+} \cdot 4\text{pic}^-$ matched well with the Pb-O distances determined by crystallography. Both coordination numbers and EXAFS Pb-O distances for $[1]_{16} \cdot 2\text{Pb}^{2+} \cdot 4\text{pic}^-$ and $\text{Pb}^{2+} \cdot \text{TBA}$ were identical, thus providing evidence that the Pb^{2+} ion was located between the two G-quartets in $\text{Pb}^{2+} \cdot \text{TBA}$. This study not only established EXAFS as a method for providing quantitative information about binding between DNA and metal ions, it also again demonstrated the value of using lipophilic G-quadruplexes containing G (**1**) as models for locating metal ions in the analogous DNA structures.

4.3. G-Quartets and IsoG Pentamers: Controlling the Self-Assembly through the Nucleobase Structure and the Cation Template

The outcome of self-assembly is determined by the information programmed into the components. Even small perturbations to those building blocks can dramatically alter supramolecular structure. Consider G (**1**) and isoG (**16**): These isomers differ only in the location of an oxygen and nitrogen atom, yet they self-assemble much differently. Crystal structures show that G (**1**) forms a hexadecameric G-quadruplex, $[1]_{16} \cdot 4\text{M}^+$,^[29–33] whereas isoG (**16**) gives a decamer, $[16]_{10} \cdot \text{Cs}^+$, in which hydrogen-bonded pentamers sandwich a Cs^+ ion.^[221,222] Comparative studies of G (**1**) and isoG (**16**) have led to two conclusions: 1) the size of the self-assembled macrocycle depends on the nucleoside's hydrogen bonding pattern and 2) a cation is needed to stabilize these synthetic macrocycles.

In accord with our studies on nucleosides in organic solvents, the research groups of Switzer and Seela have found that the nucleobase and cation play a similar role in the self-assembly of DNA. Thus, DNA oligonucleotides with d-isoG sequences form five-stranded structures in the presence of Cs^+ ions.^[223–225] Chaput and Switzer rationalized^[223] that the size of the hydrogen-bonded macrocycle is influenced by the relative location of the hydrogen bond donors and acceptors in the nucleobase (Figure 23). G-quartets form because the G donor and acceptor units are 90° to each other, thus ensuring the formation of a planar tetramer with linear hydrogen bonds. The ability of isoG to form a hydrogen-bonded pentamer, particularly in the presence of a large templating cation, was proposed to originate from the 67° angle between the van der Waals surfaces of the hydrogen bonding faces of the isoG. This angle is close to a cyclic pentamer's optimum angle of 72° ($360^\circ/5$).^[223]

In agreement with these above arguments, the identity of the cation is critical in controlling the self-assembly of isoG-

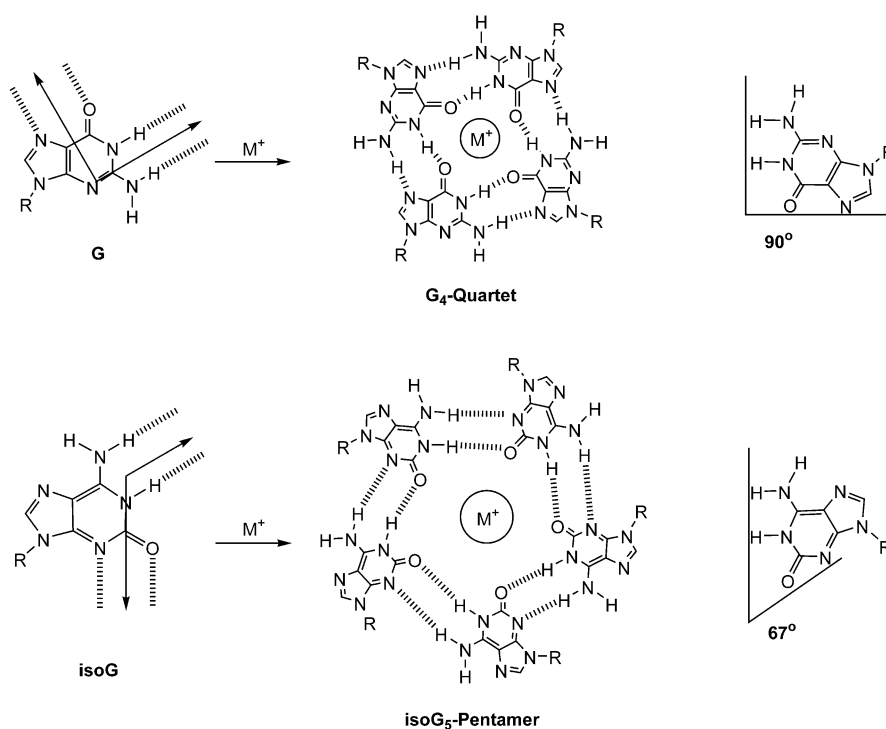


Figure 23. Self-association of G (**1**) and isoG (**16**) in the presence of cations to give hydrogen-bonded G_4 -quartets or isoG_5 -pentamers, respectively. The relative orientation of the hydrogen-bond donor and acceptor groups on the nucleoside determines the size of the assembly formed. The van der Waals angle of 67° for IsoG favors pentamers, whereas the 90° angle in G favors a quartet.^[223]

DNA. Thus, the same sequences that form pentaplexes with Cs^+ ions assemble into four-stranded structures with the smaller (and more charge dense) K^+ and Na^+ ions.^[223–228] Seela and Kröschel showed that addition of Cs^+ ions to $\text{d}(\text{T}_2\text{isoG}_4\text{T}_2)$ gave only the pentaplex, whereas only the tetraplex was identified in the presence of Na^+ ions. Remarkably, both the four-stranded and five-stranded DNA exist together in a solution containing Rb^+ ions, an ion intermediate in size between Na^+ and Cs^+ .^[225] Recent calculations have confirmed that formation of isoG quartets and isoG pentamers depends on the size of the templating cation.^[229] Since isoG and G are self-complementary,^[230,231] it is also possible that G and isoG may be able to form mixed assemblies.^[232]

In addition to the ligand and the cation, the anion may also affect the self-assembly of lipophilic nucleosides. In our early studies, we concluded that isoG (**16**) self-assembled into tetramers in the presence of potassium picrate.^[233] In retrospect, the coordinating picrate anion may have compromised the determination of the stoichiometry. Crystal structures and NMR data later obtained with the noncoordinating Ph_4B^- ion were consistent with isoG (**16**) forming hydrogen-bonded pentamers with alkali-metal cations in organic solvents.^[221,222,234,235]

Both G (**1**) and isoG (**16**) require a cation for self-assembly. This was demonstrated in ^1H NMR and ESI-MS experiments carried out during the study of the “self-sorting” of **1** and **16**.^[236] A 1:1 ratio of G (**1**) and isoG (**16**) in CD_2Cl_2 without cations gave a mixture of ill-defined hydrogen-

bonded complexes. Addition of $\text{Ba}(\text{pic})_2$ to the nucleoside mixture resulted in formation of the discrete complexes $[\mathbf{1}]_{16} \cdot 2 \text{Ba}^{2+}$ and $[\mathbf{16}]_{10} \cdot \text{Ba}^{2+}$ (Figure 24). The separate aggregation of the isomers in these experiments demonstrated the central role of the cation in expressing the hydrogen-bonding and base-stacking information embedded in these nucleosides. This self-sorting experiment with G (**1**) and isoG (**16**) provides an example of the shifting equilibrium essential for dynamic combinatorial chemistry.^[237] Wu and Isaacs have recently shown that cation-templated self-sorting of G (**1**) and isoG (**16**) even occurs in mixtures containing six other potentially competitive hydrogen-bonded assemblies.^[238]

4.4. Empty Quartets. Guanosine Self-Assembly Without a Cation

While the dogma seems to be that a cation is needed to stabilize the G-quartet, Sessler et al. have shown otherwise.^[45] A crystal structure of a G analogue **18**, modified with a large C8 substituent, revealed a hydrogen-bonded G-quartet with an empty cavity. Solution NMR spectroscopy in CD_2Cl_2 also showed characteristic signals for a stable G-quartet. The critical feature needed to obtain an “empty” G-quartet is a conformationally constrained monomer. In the absence of a cation, unconstrained guanosine derivatives self-associate to give ribbon structures by using the N3 position as a hydrogen-bond acceptor. In **18**, the dimethylaniline substituent at C8 forces the nucleoside into a *syn* conformation, where the sugar blocks the N3 position, thus preventing ribbon formation (Figure 25). Consequently, the Hoogsteen pairing that results in the G-quartet is the only option available to **18**. This study illustrates that control over monomer conformation can dramatically influence the self-assembly of guanosine.

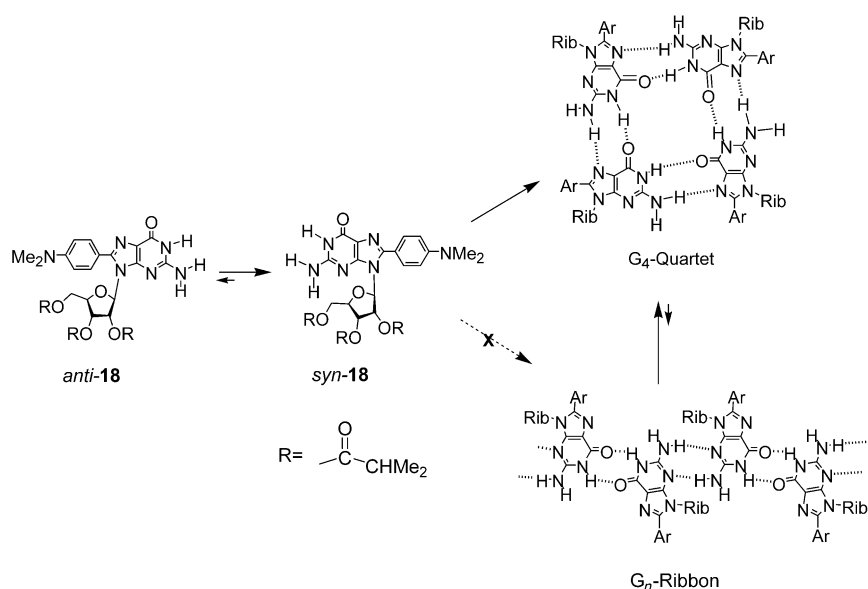


Figure 25. The conformationally constrained guanosine **18** forms a G-quartet without the need for a cation template.^[45]

More recently, Sessler et al. have prepared another cyclic hydrogen-bonded assembly from the lipophilic dinucleoside **19** (Figure 26).^[239a] This guanosine–cytosine conjugate associates through self-complementary Watson–Crick hydrogen bonds to form a cyclic trimer (**19**)₃. The use of modified nucleobases to control self-assembly, expands on efforts that have used synthetic CG analogues to build such elegant structures as Janus molecules, self-assembling dendrimers, and helical rosettes.^[239b]

4.5. Enantiomeric Self-Recognition of Lipophilic Nucleosides: Formation of Homochiral Hydrogen-Bonded Assemblies

Although not directly involved in the G-quartet hydrogen bonds, the sugar can modulate nucleoside self-assembly. We

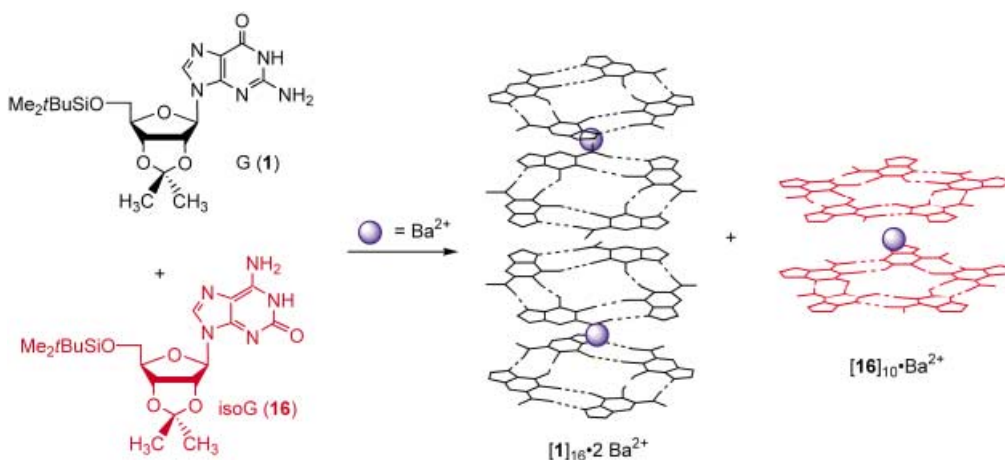


Figure 24. The isomers G (**1**) and isoG (**16**) “self-sort” in the presence of barium picrate to give the discrete complexes $[\mathbf{1}]_{16} \cdot 2 \text{Ba}^{2+} \cdot 4 \text{pic}^-$ and $[\mathbf{16}]_{10} \cdot \text{Ba}^{2+} \cdot 2 \text{pic}^-$.^[236]

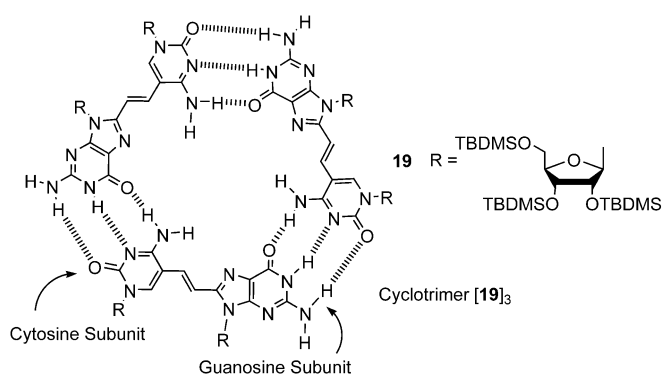


Figure 26. Self-assembly of the lipophilic dinucleoside **19** into cyclotrimer (**19**)₃.^[239a] TBDMS = *tert*-butyldimethylsilyl.

have shown that sugar chirality influences the self-assembly of guanosine. Racemic nucleosides D,L-G (**1**) and D,L-isoG (**16**) undergo highly enantioselective self-recognition.^[31,222] In both cases, an achiral metal ion triggers the stereoselective assembly.

The X-ray structure of [D-**16**]₁₀·Cs⁺·Ph₄B⁻ showed nucleobase–sugar hydrogen bonds between neighboring isoG units within a pentamer.^[222] We postulated that these sugar–base hydrogen bonds, besides contributing to the outstanding Cs⁺ selectivity of the ionophore,^[240] might also transmit information from one sugar to its base-paired neighbor. Indeed, a subsequent crystal structure confirmed that D,L-isoG (**16**) underwent enantiomeric self-recognition in the presence of CsPh₄B. The resulting “*meso*” decamer, [D-**16**]₅·Cs⁺·[L-**16**]₅·Ph₄B⁻ had one pentamer composed of only D-**16** and the other pentamer made up entirely of L-**16** (Figure 27).^[222] The Cs⁺ ion was sandwiched by the two homochiral pentamers. This “*meso*” diastereomer was also the major species in solution. We proposed that the enthalpy provided by the additional five sugar–nucleobase hydrogen bonds helps overcome the negative entropy associated with enantiomeric self-association.

Later, we identified an even more impressive example of enantiomeric self-recognition: formation of a homochiral assembly containing 16 nucleosides. In the presence of barium picrate D,L-**1** formed homochiral G-quadruplexes [D-**1**]₁₆·2Ba²⁺·4pic⁻ and

[L-**1**]₁₆·2Ba²⁺·4pic⁻,^[31] while in the presence of potassium picrate a diastereomeric mixture was obtained. Since Ba²⁺ and K⁺ have similar ionic radii, the cation's charge is clearly crucial for the high levels of enantiomeric self-recognition obtained with Ba²⁺ ions (Figure 28). This result again argues that the enthalpic contribution from self-assembly of G (**1**) around the divalent Ba²⁺ ion (with a corresponding increase in cation–dipole, hydrogen bond, and stacking energies relative to K⁺) compensates for the entropic cost of enantiomeric separation. We envision that such homochiral assemblies may be used for enantioselective separations and catalysis.

4.6. Binding of Anions to Lipophilic G-Quadruplexes to Form Ion-Pair Receptors

While it is generally appreciated that G-quartets coordinate cations, less attention has been paid to the role of the

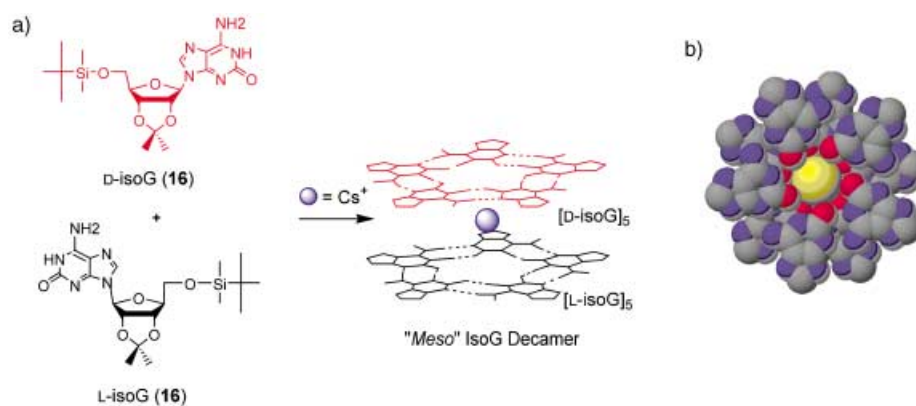


Figure 27. a) Enantiomeric self-association: the addition of Cs⁺ ions to racemic (D,L)-isoG (**16**) resulted in formation of homochiral hydrogen-bonded pentamers. The major species in solution and in the solid state is the “*meso*” decamer [D-**10**]₅·Cs⁺·[L-**16**]₅.^[222] b) Top view of the crystal structure of [D-**10**]₅·Cs⁺·[L-**16**]₅.^[222]

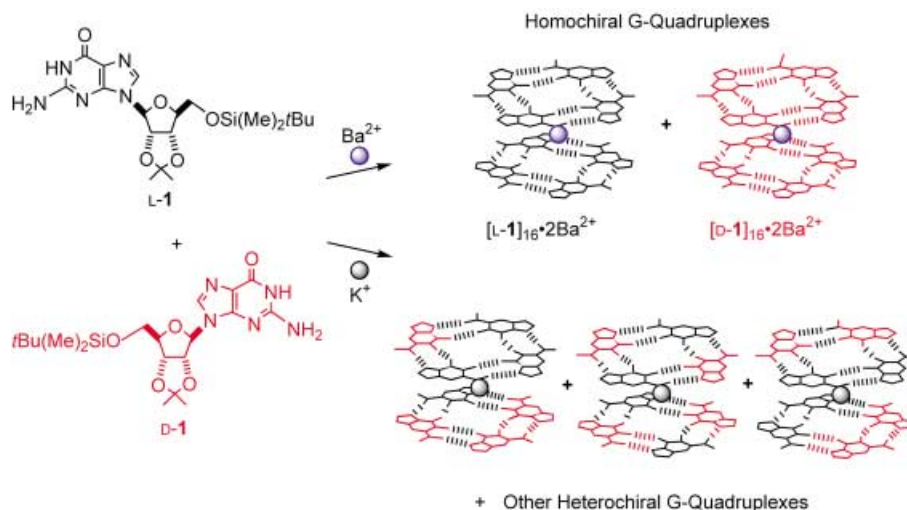
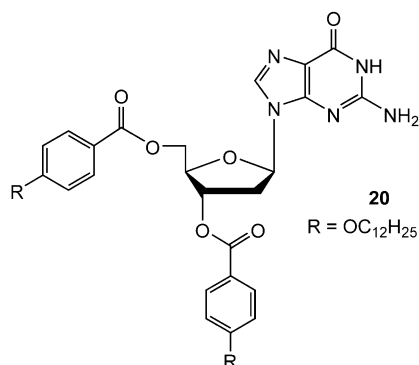


Figure 28. Cation-dependant enantiomeric self-association of G (**1**). Racemic (D,L)-G (**1**) self-assembles in the presence of Ba²⁺ ions to give homochiral G-quadruplexes [D-**1**]₁₆·2Ba²⁺·4pic⁻ and [L-**1**]₁₆·2Ba²⁺·4pic⁻. The addition of K⁺ ions to G (**1**) gave a diastereomeric mixture of heterochiral assemblies.^[31]

anion in self-assembly. For nucleotides and oligonucleotides the hydrogen-bonding ligand carries an anionic phosphate group. This is not so for nucleosides, where a counterion is needed for the G-quartet-bound cation. Our studies of lipophilic G-quadruplexes in organic solvents indicate that anions can indeed significantly influence the properties of G-quartets.

Gottarelli and co-workers discovered that the lipophilic deoxyguanosine derivative **20** extracted a K^+ salt of *N*-



dinitrophenyl-L-tryptophan from water into CDCl₃ with a 3:1 enantioselectivity over the D-Trp enantiomer.^[241] This result indicated that there were attractive interactions between anions and the chiral G-quadruplex made from **20** and K^+ .

We also found that lipophilic G-quadruplexes bind anions. Self-assembly of G (**1**) provides a structure that we regard as an ion-pair receptor.^[32,242] Most ion-pair receptors are ditopic, that is, single molecules with prefabricated cation and anion binding sites.^[243] In contrast, the allosteric ion-binding sites within the G-quadruplex are generated by a network of noncovalent interactions.^[244] Cations template G-quartets that stack so that their N2 amino groups generate hydrogen-bonding sites for anions. Much of the stability of the G-quadruplex arises from the four picrate anions that clip these inner G-quartets together (Figure 29). Moreover, the picrate

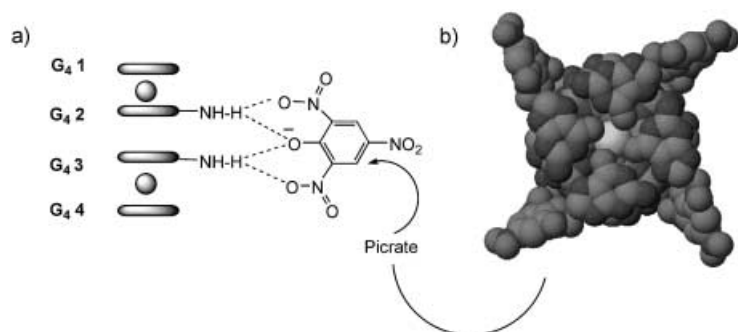


Figure 29. a) Hydrogen bonds between the nucleobase and picrate in the hexadecamer $[1]_{16} \cdot 2Sr^{2+} \cdot 4pic^-$.^[32] The amino protons are derived from the exocyclic N2 groups on the guanine. b) Top view of the G-quadruplex, with the sugars removed. The hydrogen bonds between the nucleobase and the anion result in four picrate anions forming an anionic belt around the G-quadruplex between G-quartet layers two and three.

ions remain bound to the G-quadruplex in solution and the anion can greatly modulate the kinetic stability of the structure. Thus, NMR experiments have shown that the anion controls the rate of rearrangement of $[1]_8 \cdot M^{2+}$ octamers between two different hexadecamers. For example, the formation of the “mixed” hexadecamer $[1]_8 \cdot Ba^{2+} \cdot [1]_8 \cdot Sr^{2+} \cdot 4pic^-$ from a 1:1 ratio of the Ba^{2+} and Sr^{2+} G-quadruplexes occurred 100-times slower when the anion was picrate than when the anion was SCN^- .^[32] The tridentate picrate maintains a stronger hydrogen-bonding network with the exocyclic N2 amino groups of the G-quadruplex than does a monodentate thiocyanate anion.

The rearrangement of octamers $[1]_8 \cdot M^{2+}$ can be halted altogether by increasing the basicity of the anion. Thus, with 2,6-dinitrophenolate (2,6-DNP), a much more basic anion than picrate, no isomerization product $[1]_8 \cdot Ba^{2+} \cdot [1]_8 \cdot Sr^{2+} \cdot 4(2,6-DNP)^-$ was observed even two months after combining the Ba^{2+} and Sr^{2+} G-quadruplexes in CD₂Cl₂.^[242] These studies show that noncovalent assemblies can be locked in place with other peripheral noncovalent interactions, a feature that should enable regioselective modification of G-quadruplexes.

4.7. Dynamic Exchange in Self-Assembled Ionophores

The building blocks and bound guests in noncovalent assemblies exchange with “free” components in solution (Figure 30).^[245] Quantifying such dynamic processes are important for determining self-association mechanisms.^[68] Such mechanistic insight, when coupled with structural knowledge, will surely guide the design of improved self-assembling systems.

We used 1H - 1H and 7Li - 7Li EXSY NMR (EXSY = exchange spectroscopy) as well as ^{133}Cs NMR spectroscopy to probe the dynamic properties of the decamers $[16]_{10} \cdot M^+$ in organic solvents.^[235,236] While the $[16]_{10} \cdot Cs^+$ decamer is thermodynamically stable, it is kinetically labile toward Cs^+ exchange. This dynamic ion exchange is desirable if one wants to use these complexes for ion separations and transport. A study of the exchange processes for $[16]_{10} \cdot Cs^+$ in CDCl₃ by ^{133}Cs and 1H NMR spectroscopy showed that the Cs^+ guest exchanges with free Cs^+ 40 000 times faster than the exchange between bound and “free” isoG **16**. This clear difference in the rates of exchange indicates that cation exchange occurs without complete dissociation of the hydrogen-bonded complex. The strength of the hydrogen bond in the isoG decamer in the series $[16]_{10} \cdot M^+$ ($M = Li, Na, K, Rb,$ and Cs) also depends on the identity of the cationic guest. Thus, exchange of isoG (**16**) between bound and free sites was 10–100-times slower for the Cs^+ decamer $[16]_{10} \cdot Cs^+$ than decamers containing the other alkali-metal ions. This result also highlights the extensive cooperativity of forces that exist in these self-assembled ionophores; thus, the bound Cs^+ ion increased the kinetic stability of the assembly by strengthening the hydrogen-bonding network.^[235]

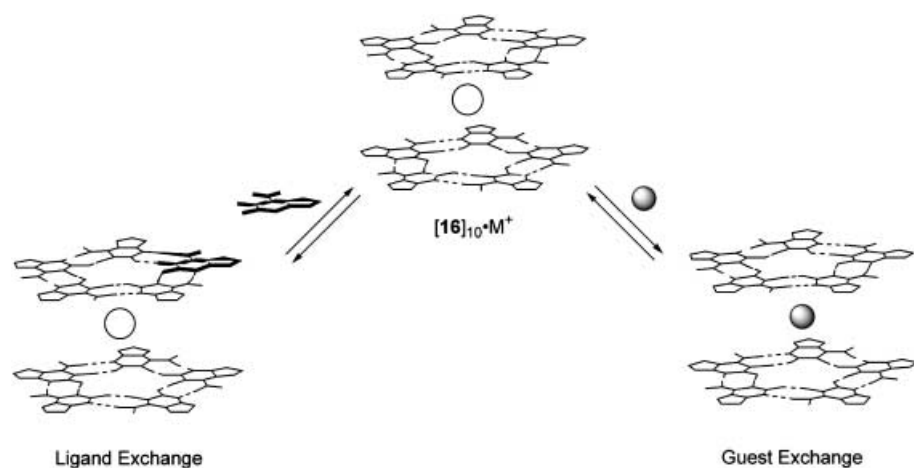


Figure 30. Dynamic exchange processes in the decamer $[16]_{10}\cdot\text{Cs}^+$. Both the isoG ligand **16** and the cationic guest Cs^+ exchange with free species in solution. NMR data shows that Cs^+ exchange is over 40000 faster than exchange of the ligand isoG (**16**).^[235]

4.8. Why Build Self-Assembled Ionophores?

Covalent ionophores such as crown ethers and calixarenes have binding sites already built into their frameworks.^[246,247] While preorganization may enable strong ion binding, the synthetic effort needed to fix the conformation is often considerable. Alternatively, noncovalent interactions can be used to synthesize self-assembled ionophores more efficiently.^[206,208,240] In addition to improved synthetic accessibility, self-assembled ionophores have other potential advantages over their covalent counterparts. In particular, it should be easy to reversibly assemble and disassemble these dynamic structures by altering the solvent, temperature, or pH.

The radionuclide $^{137}\text{Cs}^+$ is a major contaminant in nuclear waste.^[248] One challenge in $^{137}\text{Cs}^+$ separation is achieving sufficient selectivity, as the waste contains more Na^+ and K^+ than Cs^+ ions. The selective extraction of Cs^+ ($r = 1.67 \text{ \AA}$) in the presence of Na^+ ($r = 0.97 \text{ \AA}$) and K^+ ions ($r = 1.33 \text{ \AA}$) is challenging because of its large size. The larger crown ethers ([21]crown-7 and [24]crown-8 derivatives) usually have only modest Cs^+ selectivity because of their flexibility.^[249–252] Better results are obtained using more rigid macrocycles such as the calix[4]arene–crown ethers.^[253,254] While their Cs^+ binding constants and Cs^+/M^+ selectivity are often impressive, these macrocycles can be expensive to synthesize. As mentioned above, isoG (**16**) can bind Cs^+ with high affinity and selectivity with the formation of a hydrogen-bonded decamer $[16]_{10}\cdot\text{Cs}^+$

in the solid state and in solution (Figure 31).^[221,222] In competition experiments the self-assembling isoG (**16**) was able to quantitatively remove bound Cs^+ ions from a calix[4]arene–crown ether.^[240] The self-assembled ionophore formed from isoG (**16**) is one of the strongest and most selective Cs^+ ionophore identified to date.

The lipophilic decamer $[16]_{10}\cdot\text{Cs}^+$ also enables selective transport of Cs^+ ions across organic membranes. Lamb and co-workers showed that this assembly transports CsNO_3 across bulk liquid membranes (BLM) and polymer inclusion membranes (PIM) with excellent Cs^+ selectivity and Cs^+ transport rates (Table 3).^[255] The Cs^+/Na^+ transport selectivity for isoG (**16**) in the PIM experiments was consistently above 5000:1, and approached 10000:1 with 6 mM concentrations of $[16]_{10}\cdot\text{Cs}^+$. In addition, both the Cs^+ transport rate and selectivity of

the self-assembled decamer $[16]_{10}\cdot\text{Cs}^+$ were competitive with calix[4]arene–crown ethers, compounds which are currently being evaluated as extractants for nuclear waste remediation.^[255,256] These membrane transport results with $[16]_{10}\cdot\text{Cs}^+$ are certainly encouraging for the use of self-assembled ionophores in environmental separations and waste remediation.

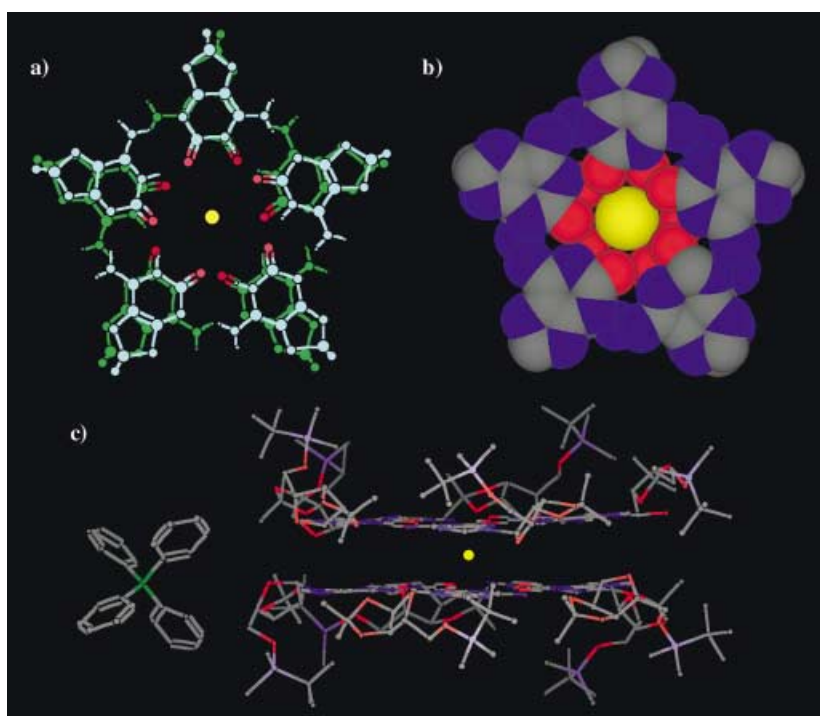


Figure 31. Crystal Structure of $[16]_{10}\cdot\text{Cs}^+\cdot\text{Ph}_4\text{B}^-$: a) Top view (the sugars have been removed for clarity); one isoG pentamer is shown in blue and the other in green. b) Space-filling model; the Cs^+ ion is bound to 10 carbonyl oxygen atoms, with a mean $d_{\text{Cs-O}} = 3.40 \text{ \AA}$. c) Side view (some of ribose atoms have been removed for clarity); this view shows the two planar isoG pentamers sandwiching the encapsulated Cs^+ ion.^[221]

Table 3: Cs⁺ selective ionophore. Percentage and permeability of transported cations and selectivity (Cs⁺/Na⁺) through polymer inclusion membranes containing precomplexed [16]₁₀·CsBPh₄.^[a,b]

c[16] ₁₀ [mM]	Transport [%]		Permeability [m s ⁻¹]		Selectivity (Cs ⁺ /Na ⁺)
	Cs ⁺	Na ⁺	Cs ⁺	Na ⁺	
1.1	16.5	0.002	2.1 × 10 ⁻⁷	2.8 × 10 ⁻¹¹	7500
2.1	31	0.004	4.4 × 10 ⁻⁷	8.3 × 10 ⁻¹¹	5300
4.1	44.5	0.004	6.4 × 10 ⁻⁷	8.3 × 10 ⁻¹¹	7700
6.2	53	0.004	8.0 × 10 ⁻⁷	8.3 × 10 ⁻¹¹	9600

[a] Transport conditions: Source phase: 1.0 mM CsNO₃ + 50 mM NaNO₃ in water. Carrier: [16]₁₀. Receiving phase: 0.5 M HNO₃. Total transport time 24 h. [b] Values taken from reference [255].

4.9. Toward Synthetic Ion Channels

Lipophilic G-quartets are promising scaffolds on which to build functional assemblies. With an eye toward artificial photosynthesis, Masiero et al. described a lipophilic guanosine carrying a porphyrin chromophore.^[257] They obtained CD and NMR evidence for the formation of a supramolecular complex containing a G₈·K⁺ octamer with an array of eight porphyrins.

Our research group is interested in using the G-quadruplex to build transmembrane ion channels. NMR studies have shown that base pairs in DNA G-quadruplexes open slowly and G-quadruplex dissociation is often quite slow, taking days or weeks. Yet, K⁺ ions are bound for only milliseconds.^[258] These results suggest that ions move without disruption of the G-quartet, thus making the G-quadruplex analogous to an ion channel. For example, Feigon and co-workers showed that NH₄⁺ ions enter and exit through the

ends of a G-quadruplex, which prompted the authors to also suggest that such structures mimic ion channels.^[212]

Among various designs,^[259–261] the G-quartet has been proposed as a scaffold on which to build synthetic ion channels.^[29,262,263] Our efforts involved self-assembly of **21**, a calix[4]arene with four attached guanosine moieties (Figure 32).^[263] The 1,3-alternate conformation of the calixarene orients orthogonal pairs of G nucleosides for intermolecular G-quartet formation upon addition of a cation. We reasoned that a tubular structure, with a channel of alternating G-quartets and calixarene macrocycles, would result if the G units on both sides of **21** were to self-associate upon cation templation. The addition of NaBPh₄ to a solution of **21** gave a noncovalent polymer that precipitated from solution. Electron microscopy studies showed that the solid consisted of micrometer-long strands and bundles (with diameters of 3 and 50 nm, respectively). Since a G-quartet has a diameter of 2.5 nm,^[29] the single strands had dimensions approaching that of a G-quartet. This noncovalent polymer dissolved upon heating or acidification, thus indicating that 1) hydrogen-bond assembly can control long-range organization and 2) self-assembly of **21** is reversible. We are currently testing whether **21** and analogues can act as synthetic ion channels.

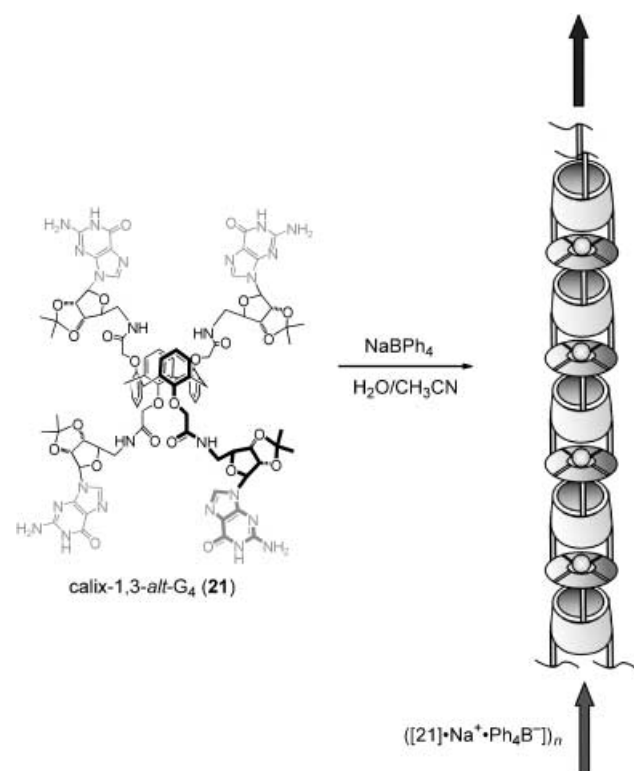


Figure 32. The guanosine–calix[4]arene-1,3-*alt* **14** self-associates in the presence of alkali-metal cations to form a 1D nanotube, presumably formed by a G-quartet-based polymer.^[263]

5. Guanosine Self-Assembly in Materials Science, Biosensor Design, and Nanotechnology

“Quadruplexes show promise as components for nanowires, ion channels, and building blocks for directing the assembly of nanoscale components into sophisticated structures.”

M. Keniry^[10]

The burgeoning field of nanotechnology depends on controlled positioning of molecules on the nanometer to micrometer scale. The four-stranded G-quadruplex seems ideal for ordering material over such distances. Self-assembly is a powerful way to arrange molecules into specific patterns and DNA has been used to construct nanoscale devices.^[264,265] Efforts in using guanosine derivatives to build supramolecular structures with new properties are described below.

5.1. DNA Nanostructures: G-Wires, Frayed Wires, and Synapses

Fiber diffraction studies on crystalline GMP and poly-(guanylic acid) had shown that these compounds self-assem-

ble into rods of stacked G-quartets.^[2,39–41] It was later discovered that DNA superstructures formed from sequences with 3'-terminal dG residues. Sen and Gilbert reported that $d(A_4G_4)$ gave stable assemblies that were much larger than expected for a four-stranded G-quadruplex $[d(A_4G_4)]_4$.^[266] Although the authors noted that the sequences could aggregate in different ways, the G-quartet cores favored a head to tail orientation to give a continuous polymer, with poly-A tails radiating from the core. Guo and co-workers also observed the formation of superstructures upon cation-templated aggregation of $d(A_4G_4)$.^[267]

In the mid-1990s, the research groups of both Henderson and Sheardy further established that G-rich DNA formed nanometer assemblies.^[268–271] They showed by using gel electrophoresis that $d(G_4T_2G_4)$, in the presence of K^+ and Mg^{2+} ions formed high molecular weight assemblies that were well resolved from the smaller G-quadruplex $[d(G_4T_2G_4)]_4$. The polymers were extraordinarily stable; heating at 80 °C or dissolving in 8 M urea did not denature them. Marsh and Henderson coined the term “G-wires” to describe the continuous, parallel-stranded DNA superstructures formed when the 5'-end of one DNA duplex with G-G pairs associates with the 3'-end of a similar duplex (Figure 33).^[268] Atomic

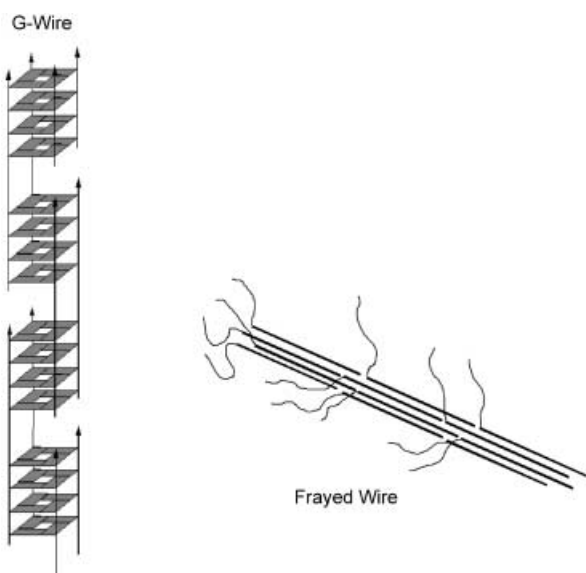


Figure 33. Superstructures formed by G-rich oligonucleotides. “G-wires” are formed by association of $d(G_4T_2G_4)$ ^[263] while “frayed wires” are formed by association of $d(G_{15}A_{15})$. The G residues make up the core, while the A_{15} arms radiate from the core.^[273]

force microscopy showed that G-wires formed from $d(G_4T_2G_4)$ were 10–1000 nm in length and 18–25 Å in width, which is consistent with the 25-Å diameter of the G-quartet.^[269] The influence of the cation on polymer structure and stability was also consistent with formation of a G-quartet, as addition of Na^+ ions gave longer G-wires that were much less compressible than duplex DNA on mica. Marsh and Henderson proposed that G-wires could be useful in nanotechnology, nanoelectronics and biosensor development.

Chen showed that certain G/C sequences formed cross-linked G-wires under acidic conditions.^[272] He proposed that the sequence CGG used C-CH⁺ base pairs to bridge G-wires into a dendrimeric structure.

MacGregor and co-workers identified DNA sequences, $d(A_{15}G_{15})$ and $d(T_{15}G_{15})$, that give extended structures in the presence of K^+ ions.^[273–277] They called these structures “frayed wires” to distinguish them from G-wires (Figure 33). The unique feature of frayed wires is the flexible A_{15}/T_{15} strands that radiate from a guanine core (akin to barbed wire). The fact that the N7 position of guanosine can be alkylated without loss of structure suggests that typical G-quartets do not form the core. Extensive 2D aggregation was observed by AFM when the single-stranded T_{15} complement was added to a solution of frayed $d(A_{15}G_{15})$ wires.^[278] One experiment that shows potential applications for frayed wires involved attachment of enzymes to the A_{15} arms. A network formed by the noncovalent attachment of avidin-conjugated peroxidase to the frayed wires with biotinylated arms retained its structure and enzyme activity.^[278]

Sen and co-workers described a method to build nanostructures based on the synapsis between two DNA duplexes.^[279–281] Each duplex contained “synaptic domains”, that is, repeating G-G base pairs engineered to facilitate formation of a G-quartet upon dimerization of the duplex (Figure 34). An attractive feature of synapsis is that it doesn't require annealing. The addition of cations to a DNA duplex triggers G-quartet-mediated dimerization to give the nanostructures. The yields of synapsed DNA follow the trend typically observed for cationic G-quartet stabilization ($K^+ > Rb^+ > Na^+ > Li^+, Cs^+$ and $Sr^{2+} > Ba^{2+} > Ca^{2+} > Mg^{2+}$), which led to the statement: “Guanine-mediated synapsis ... provides a means for the self-recognition and supramolecular assembly by intact, unmelted DNA double helices under conditions of low temperature and physiological salt.”^[280]

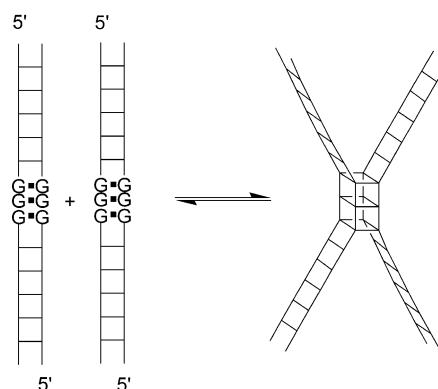


Figure 34. “Synapsed” DNA formed from G-quartet structures between duplex DNA strands containing G-G mismatches.^[280]

5.2. Formation of Biosensors and Nanomachines with G-Quadruplex DNA

While DNA aptamers have potential as therapeutics and diagnostics (see Sections 3.6 and 3.7), they also have appli-

cations in bioanalytical chemistry. Small molecules and proteins can be separated by using G-quadruplex DNA as stationary phases in chromatography or electrophoresis. McGown and co-workers found that G-quadruplex DNA based on the TBA sequence can bind nontarget compounds with useful selectivity.^[282–284] The authors noted that these DNA aptamers are attractive stationary phase materials, as a consequence of their ease of synthesis and surface attachment as well as their ability to change binding affinities by sequence modification. These G-quadruplex aptamers effected baseline separation of the enantiomers of D,L-Trp and D,L-Tyr, as well as an aromatic hydrocarbon mixture.^[282] In another study, DNA aptamers featuring an intramolecular G-quadruplex served as the stationary phase for the separation of the isomeric dipeptides Trp–Arg and Arg–Trp.^[283] In addition to the separation of small molecules, a G-quadruplex aptamer was used for electrochromatographic separation of bovine β -lactoglobulins A and B, proteins that vary by just 2 of 162 amino acids.^[284]

DNA G-quadruplex aptamers labeled with fluorescent dyes have also served as a prototype for biosensors.^[285–289] In particular, FRET has been used to study the secondary structure of G-rich DNA oligonucleotides.^[290,291] FRET is a distance-dependent method for detecting conformational changes over distances of 10–100 Å. Fluorescence is quenched when a donor fluorophore and an acceptor chromophore are close in space, but when the distance between the donor and acceptor increases, as a result of a conformational change, there is a corresponding increase in fluorescence. Intramolecular folding of an oligonucleotide into its G-quadruplex form leads to FRET between donor and acceptor dyes that are attached to the 5' and 3' ends. This sensitive technique allows the effect of cations, ligands, nucleic acids, and proteins on the equilibria of unfolded and G-quadruplex DNA to be monitored. For example, Takenaka and co-workers showed that a single-stranded DNA folded into an intramolecular G-quadruplex can detect K^+ ions in water at submicromolar concentrations.^[292]

FRET has also been used to monitor the formation of molecular “nanomotors” from single-stranded DNA.^[293,294] Li and Tan used FRET to monitor in real time the conformational switching between a double-stranded duplex and a folded G-quadruplex caused an extension and shrinking motion.^[293] In a closely related study, Alberti and Mergny also reported that the conformational equilibrium between DNA duplex and quadruplex defines a nanomolecular machine.^[294] The conformational states of a 21-mer DNA with a 5'-fluorescein donor and a 3'-rhodamine acceptor were detected by FRET (Figure 35). The switching between the folded unimolecular G-quadruplex and a duplex conformation caused a displacement of 5–6 nm. The machine was cycled between its closed G-quadruplex state and open duplex state by sequential addition of other DNA strands: a “C-fuel” and a “G-fuel”. The C-fuel unfolds the unimolecular G-quadruplex to generate a duplex, while the G-fuel is used to liberate the labeled 21-mer so that it refolds into a G-quadruplex.

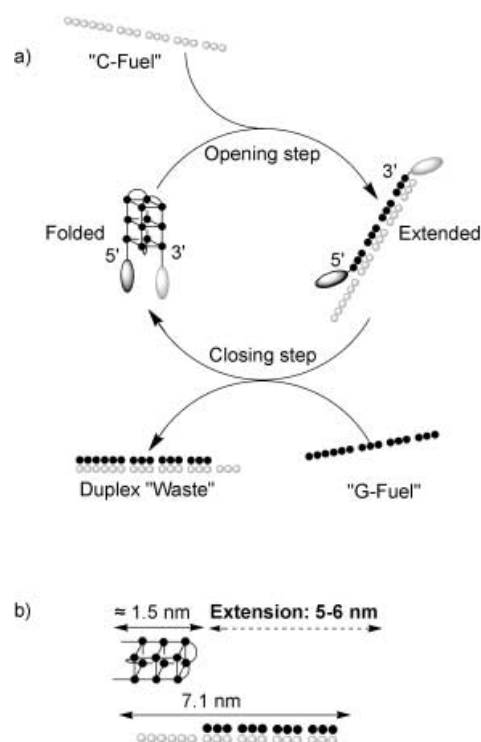


Figure 35. A G-quadruplex nanomachine.^[294] The experimental design is similar to a system described by Li and Tan.^[293] a) Switching between an intramolecular quadruplex (left) and a duplex (right). Folding of a G-rich oligonucleotide gives an intramolecular quadruplex that undergoes efficient FRET between bound fluorescein and rhodamine groups (ovals). The “C-fuel” strand is complementary to the G-rich sequence. b) Induced movement upon G-quadruplex folding and unfolding.

5.3. Dynamic Materials from Guanosine and Folate Self-Assembly

In addition to G-rich oligonucleotides, smaller guanosine derivatives also show promise for nanotechnology and materials applications. In particular, lipophilic guanosine and folate analogues form dynamic liquid crystals and gels whose morphologies can be controlled by varying the ionic conditions, solvent, or temperature.

The research groups of Rabe and Gottarelli used scanning force microscopy to show that surface changes modulate the structure of the hydrogen-bonded assembly.^[295,296] Lipophilic dG (**17**) formed films with the signature of cyclic G-quartets on mica containing bound K^+ ions. However, if the K^+ ions were washed off the surface, dG (**17**) formed extended ribbons consistent with hydrogen-bond patterns observed previously in solution and the crystalline state.^[297,298] It was shown that without cations lipophilic guanosines such as dG (**17**) form liquid crystals and gels in organic solvents. The authors showed by using electrospray mass spectrometry, solution NMR spectroscopic, and single crystal X-ray analysis that these guanosine derivatives adopt two different hydrogen-bonded structures (Figure 36).^[295,296] One of the hydrogen-bonded structures has an overall dipole (ribbon A), while the other hydrogen-bonding arrangement gives a 2D structure without a molecular dipole (ribbon B). The type of ribbon

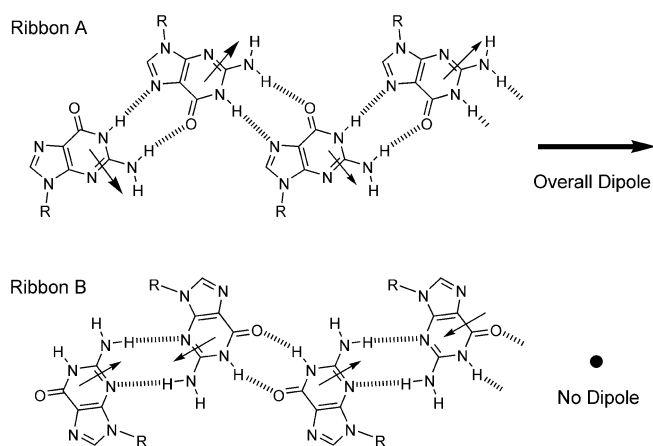


Figure 36. Two different H-bonded ribbons formed by self-assembly of lipophilic dG (**17**). Ribbon A has a dipole moment, whereas ribbon B does not.^[295,296] R = modified deoxyribose.

formed (ribbon A versus ribbon B) can be controlled by the sugar unit on the nucleoside and by the solvent. Some of these lipophilic guanosine derivatives are promising gelators, which are able to immobilize organic solvents at critical concentrations as low as 2–4 wt %.^[299]

Folic acid **22** and guanosine have similar hydrogen-bonding patterns, so it was expected that

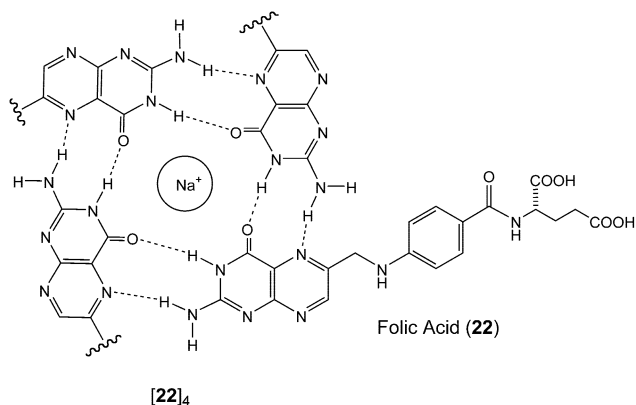


Figure 37. Self-assembly/self-organization of lipophilic folate derivatives.^[303] Ion-responsive self-assembled liquid crystals are formed on reaction with ions. The ribbon structures of a folic acid derivative undergo an ion-templated transformation to discotic tetramers.

folates would also form cyclic tetramers. Over 10 years ago Gottarelli and co-workers demonstrated that folic acid underwent a cation-dependent assembly in water.^[300] Potassium folate formed liquid crystals that were hexagonally packed columns of stacked folate tetramers even at concentrations as low as 1 wt %.^[301,302] Kato and co-workers further investigated the cation-templated assembly of lipophilic folates in organic solvents in which they focused on controlling their dynamic assembly and their materials properties.^[303,304]

A liquid crystal is a fluid somewhere between the ordered crystal and the disordered liquid.^[305] Materials that form liquid crystals without solvent are thermotropic liquid crystals, and phase transitions occur with temperature changes.

Most thermotropic liquid crystals arise from rod-shaped compounds with an aromatic core and flexible side chains. These materials form different types of liquid crystals, such as the nematic, smectic, and discotic phases. The nematic phase is a fluid with one-dimensional order along a single molecular axis, but is otherwise disordered. In smectic phases, ordered molecules are arranged in layers and there is short-range positional order within these layers. Discotic liquid crystals have even longer range positional ordering of the cyclic cores. In particular, hydrogen-bonded assemblies have been used to generate discotic liquid crystals.^[306,307]

Kato and co-workers found that temperature and cations induce a phase change in folate-derived liquid crystals. X-ray diffraction and IR spectroscopy studies indicated that lipophilic folates with 2-(3,4-dialkoxyphenyl)ethyl substituents formed thermotropic liquid crystals (Figure 37).^[308] Two liquid-crystal phases are formed depending on the conditions: a smectic and a columnar discotic phase. Similar to dG (**17**), the pterin rings of the folate hydrogen bond to give a self-

associated ribbon structure, which is the basis for the smectic phase. Alternatively, the folate ring forms a cyclic tetramer in the presence of a cation, which leads to a columnar discotic liquid crystal. The smectic phase was transformed into the hexagonal discotic phase upon addition of alkali-metal salts.^[309] The solvent polarity also induced a reorganization of the liquid crystal from ribbons to disks, as less polar solvents favor the cyclic hydrogen-bonded form.^[310] The finding that cations and solvent can trigger rearrangement from an H-bonded ribbon to cyclic tetramer indicates that folate-derived liquid crystals are dynamic materials, which are able to change their structure and properties in response to an external stimulus.

5.4. Organic Semiconductors from Hydrogen-Bonded Guanosines

In 1975 Aviram and Ratner proposed that organic molecules could conduct charge.^[311] Today, with the minia-

turization of semiconductor components, the potential of molecular electronics is even clearer.^[312] Recent experiments with DNA suggest that biomolecular self-organization is a viable strategy for electron transport.^[313,314]

Guanine derivatives are attractive candidates for electron transport. Guanine has the lowest oxidation potential of the nucleobases and it forms a variety of ordered structures. Rinaldi et al. recently fabricated a semiconductor device with dG (**17**) bridging electrodes.^[315] Atomic force microscopy showed that evaporation of a solution of dG (**17**) in CHCl₃ gave hydrogen-bonded ribbons on the surface of the device which were consistent with previous AFM and diffraction data. Current–voltage (*I*-*V*) measurements on this device revealed high conductivity when the electrode spacing was less than 100 nm.^[315,316] Asymmetric *I*-*V* curves, which are characteristic of molecular rectification, were attributed to the dipole of the hydrogen-bonded ribbon upon self-association of dG (**17**; ribbon A in Figure 36). Ab initio calculations indicated that conduction through dG (**17**) occurs by orbital overlap of stacked ribbons and not through the intermolecular hydrogen bonds.^[317,318] A major challenge is to orient these crystalline materials so that they can conduct current over longer distances. Molecular self-assembly of lipophilic dG analogues could then be coupled with lithography to form useful devices.

6. Summary and Outlook

This Review has touched on diverse areas in chemistry where the G-quartet motif is important. Three decades after its identification in 5'-GMP gels, the G-quartet really gained visibility because of the biological implications of G-quadruplex DNA. Consequently, structural characterization of nucleic acid G-quadruplexes has accelerated over the past decade. In 1992 the Protein Data Bank showed just two entries for G-quadruplexes. Ten years later, there are more than 35 deposited G-quadruplex structures, including 9 crystal structures. This data provides a wealth of information about the noncovalent interactions that drive self-assembly of G-rich nucleic acids. More structures of G-quadruplex–protein and quadruplex–ligand complexes are needed in the future; indeed, the first three examples were published in just the last year.^[59,190,191] Detailed information about protein and ligand complexes with G-quadruplexes will help us better understand G-quadruplex molecular recognition and further enhance drug design.

The most prominent role of the G-quartet relates to its potential to mediate crucial *in vivo* processes, particularly those associated with telomere structure and function. Telomerase is a key anticancer target and research is intense. A citation search of the Web of Science showed just 7 references to this enzyme in 1990, whereas more than 750 references to telomerase occurred in 2002. The development of G-quadruplex interactive drugs to inhibit telomerase will surely continue. The major challenge to getting candidates into clinical trials will be to identify nontoxic drugs that are truly selective for the G-quadruplex.^[178]

There are also many nonmedicinal aspects of G-quartets. Supramolecular chemistry has taught us much about using noncovalent interactions to build large and functional assemblies. As nanotechnology develops there will be a growing interest in using such noncovalent structures for specific functions. Of course, nature often provides useful blueprints. The G-quartet is a prime example of a natural system that is amenable to synthetic modifications and which can be used to study the structure and dynamics of molecular self-assembly. It can also inspire the design of new and useful synthetic assemblies. The use of the G-quartet and related motifs to form self-assembled ionophores, synthetic channels, dynamic liquid crystals, noncovalent polymers, nanomachines, biosensors, therapeutic aptamers, chromatography supports, new PNA assemblies, and molecular electronic devices demonstrates how concepts from biology and supramolecular chemistry can merge quite nicely.

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