Award Accounts

The Chemical Society of Japan Award for Creative Work for 2007

Designable DNA Functions toward New Nanobiotechnology

Naoki Sugimoto

Department of Chemistry, Faculty of Science and Engineering, and Frontier Institute for Biomolecular Engineering Research (FIBER), Konan University, 8-9-1 Okamoto, Higashi-Nada-ku, Kobe 658-8501

Received June 23, 2008; E-mail: sugimoto@konan-u.ac.jp

DNA has many properties that cannot be attained with other molecules. The ability of DNA to form base pairing and its structural polymorphism allow the formation of distinct secondary and tertiary structures and may have further catalytic or aptamer function. It is also of great advantage that the base pairing of a short oligonucleotide sequence can be well predicted and designed with the thermodynamic parameters of Watson–Crick base pairs, mismatch pairs, and noncanonical base pairs based on the nearest-neighbor model. We have investigated the thermodynamics of various types of oligonucleotide structures, and obtained the nearest-neighbor parameters for Watson–Crick base pair formations and fundamental information regarding the nucleotide interactions. The data of the DNA interaction energy were also applied for molecular design of a DNA logic gate and DNA nanowire. I will further discuss the importance of quantitative data of DNA interaction energy toward the rational design of artificial DNAs carried out by our laboratory, such as base pair-mimic nucleosides and DNA containing bipyridine units, useful as nanobiodevices and nanobiomaterial.

1. Extraction of "Quantity" from the Quality of DNA

1.1 DNA toward the Nanobio-Research Field. DNA has excellent properties applied to technology uses, such as the recognition of target sequences, self-assembly into defined structures, and catalytic or aptamer functions. Oligonucleotides have been examined for constructing functional molecules, because they can be synthesized by an automated synthesizer and are available commercially at a relatively low cost. Association of oligonucleotides further constructs large-sized complexes and their noncovalent interaction has an advantage of reversible assembly. Moreover, conjugation of a DNA strand with other functional materials including other biomolecules, fluorescent dyes, and metal plate surfaces or nanoparticles allows an expansion of the DNA function. Therefore, DNA is one of the most promising nanobiomaterials suitable as nano-scale materials, nano-sized devices, and medicinal and therapeutic uses. In fact, development of nanobiomaterials that apply the properties of DNA to nanotechnology have become of broader interest over the past several years.[1–4]

The most important property of DNA is the ability to form base pairing mediated by interbase hydrogen bonds. The major structure of DNA is the double helical structure of Watson–Crick base pairs. Figure 1 shows the famous Watson–Crick base pairs, of which the purine (A or G) and pyrimidine (C, T, or U) nucleotides associate with each other in accordance to the geometry of hydrogen donors and acceptors on the bases. All the nucleotide bases adopt an anti configuration at the glycosidic bond in a right-handed antiparallel-stranded duplex, and the structural isomorphism among A/T, A/U, and G/C base pairs ensures the distance between glycosidic bonds of approximately 1 nm. The base stacking interaction is important for the integrity of the duplex. The stacking interaction does not require a particular partner, because the base stacking originates from van der Waals force, dipole–dipole attraction, and hydrophobic interaction although the details of the interaction are still unclear. As a consequence of the interbase hydrogen bonds and base stacking, a typical DNA duplex adopts the B-form, while an RNA duplex usually adopts a slightly different configuration called the A-form due to the presence of a 2′-hydroxy group on ribose.

Investigation of thermodynamic aspects of the base pairing is essential to understand secondary and tertiary structures of DNA and RNA. In earlier studies, it had been assumed that the number of interbase hydrogen bonds determines the stability of Watson–Crick base pairs, that is, the three hydrogen-bonded G/C base pair is more stable than the two hydrogen-bonded A/T base pair.[5] But the nearest-neighbor model is currently widely used to account for the thermodynamic properties of Watson–Crick duplexes.[6] The model assumes the formation of a base pairing mostly affected by the nearest-neighbor (adjacent) base pair already formed, that consider the contribution of the base stacking interaction as well as the hydrogen bonds. Experimentally, the thermal stability of a nucleotide duplex is context-dependent, and it is evident that the duplex stability can be well accounted for by the sum of thermodynamics of individual nearest-neighbor interactions. The nearest-neighbor parameters of ΔH°, ΔS°, and ΔG°_{37} (a change in

Published on the web January 14, 2009; doi:10.1246/bcsj.82.1

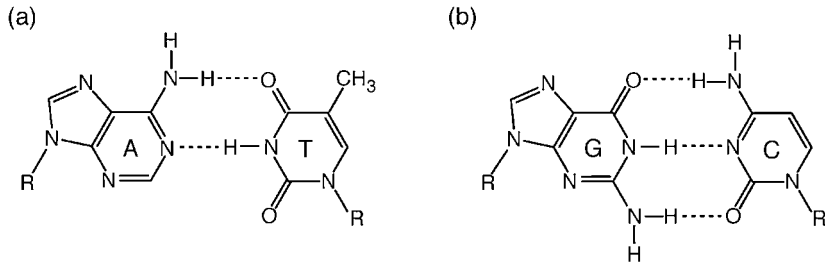


Figure 1. (a) A/T and (b) G/C Watson–Crick base pairs. R indicates the C1′ position of deoxyribose. All the bases adopt an anti configuration in an antiparallel orientation.

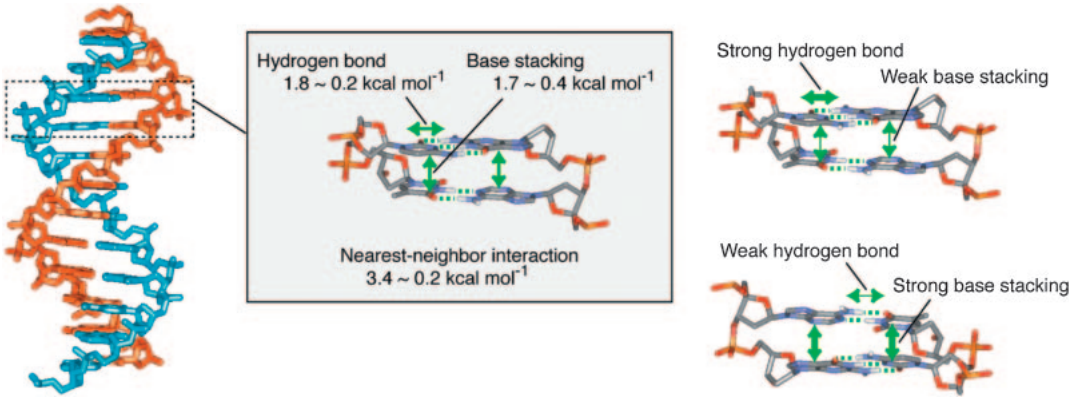


Figure 2. The $-\Delta G^{\circ}_{37}$ values of the interbase hydrogen bonds, base stacking interaction, and the nearest-neighbor interactions of a nucleotide duplex. The image of the competitive relationship between the interaction energies of hydrogen bonding and base staking is also indicated.

the Gibbs free energy at 37 °C) for Watson–Crick base pair formations have been reported,[7] and one can readily predict the thermodynamic parameters of a Watson–Crick duplex and secondary structures of any nucleotide sequence by using the nearest-neighbor parameters.

As for the formation of a Watson–Crick duplex, the hydrogen bonds and the base stacking are formed simultaneously and give the stabilization energies ($-\Delta G^{\circ}_{37}$) in the range from 3.4 to 0.2 kcal mol^{-1} (1 kcal = 4.184 kJ) in accordance to the nearest-neighbor model.[7] Investigations of the individual contribution of these interactions on the overall base pairing stability indicated comparable $-\Delta G^{\circ}_{37}$ values for forming a single hydrogen bond (1.8 to 0.2 kcal mol^{-1}) and the stacking interaction (1.7 to 0.4 kcal mol^{-1}).[8–10] Importantly, competitive correlation between the interaction energies are suggested, such that the base pair with greater stacking energy provides smaller hydrogen-bond energy, and vice versa (Figure 2).[8] This observation can be explained from their interaction geometry such that the geometry optimized for the base stacking is not suitable for the hydrogen bonding.

1.2 Interaction Energies of the RNA/DNA Duplex. In 1995, we reported the thermodynamic parameters for RNA/DNA duplexes, that was the first report of the complete nearest-neighbor parameters for the hybridization energy of RNA with DNA.[11] The obtained nearest-neighbor parameters exhibited context-dependent $-\Delta H^{\circ}$, $-\Delta S^{\circ}$, and $-\Delta G^{\circ}_{37}$ values ranging from 16.3 to 5.5 kcal mol^{-1}, 47.1 to 12.3 cal mol^{-1} K^{-1}, and 2.9 to 0.2 kcal mol^{-1}, respectively. The impact of the parameters is the applicability for the prediction of the interaction energy of RNA/DNA hybrid duplexes formed during RNA transcription and for the sequence design of an antisence DNA. In the study, we found that when the nucleotide sequence is identical, an RNA duplex is more stable than a DNA duplex and an RNA/DNA duplex, and which is more stable between a DNA duplex and an RNA/DNA duplex is determined by the sequence context. Notably, this finding disagrees with the structural aspects of an RNA/DNA duplex that often adopts an A-form or A-like form like an RNA duplex (Figure 3). Accordingly, it is probable that the helical configuration is not the major determinant of the duplex stability.

To investigate the origin of superior stability of an RNA duplex, we investigated the role of the 2′-hydroxy group on the duplex stability by using chimeric RNA–DNA strands.[12] The 2′-hydroxy group affects the sugar puckering, hydration, and intrastrand hydrogen bonds with sugar or phosphate oxygen atoms.[13] We found that the 2′-hydroxy group provided a substantial stabilization energy and the hydroxy group on the 5′-side of the chimeric junction affected the nearest-neighbor interaction at the chimeric junction. It was thus concluded that the 2′-hydroxy group of ribose provided extra interaction energy and increased the stability of the nearest-neighbor interaction, which at least partly explains the superior stability of an RNA duplex to a DNA duplex.

We also investigated several types of single mismatches formed in an RNA/DNA duplex and proposed their prediction parameters.[14] With the nearest-neighbor parameters for the mismatch formations, one can now predict the thermodynamic parameters of RNA/DNA duplexes even containing a single mismatch. Among the mismatches, rG/dT, rU/dG, and rG/dG mismatches were relatively stable, in agreement with the

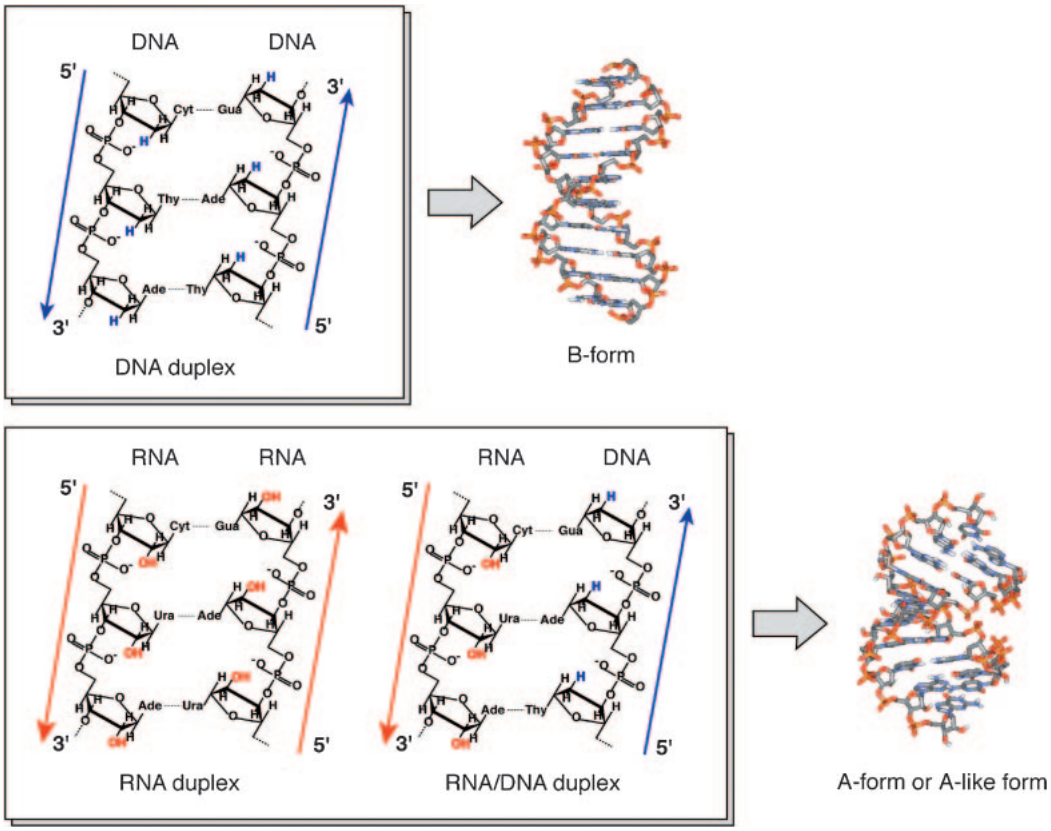


Figure 3. Comparison of typical helical structures of DNA duplex, RNA duplex, and RNA/DNA duplex.

formation of two hydrogen bonds. Studies with rG/dT and rU/dG mismatches in an RNA/DNA duplex revealed that that the C5-methyl group of thymine paired with rG increased the duplex stability by 0.5–0.1 kcal mol^{-1}. In addition, the 2′-hydroxy group of ribouridine paired with dG stabilized the duplex by 0.6 kcal mol^{-1}. The stabilization provided by the 2′-hydroxy group of ribose is consistent with the greater stability of an RNA duplex than a DNA duplex. We also compared the thermodynamic parameters of the rU/dG and rG/dT mismatches with those reported for DNA (G/T mismatches) and RNA duplexes (G/U mismatches).[15,16] It was an intriguing finding that the relatively stable mismatches in RNA/DNA showed stability similar to the G/U mismatches in an RNA duplex, while unstable RNA/DNA mismatches showed stability similar to the G/T mismatches in a DNA duplex. The observation implies a stability–structure relationship in the RNA/DNA duplex. Other mismatch pairs such as A/A and C/T mismatches that do not fit in a duplex showed relatively low stability.[14]

1.3 Structural Polymorphism of DNA. Although the major conformation of DNA is the duplex structure by the Watson–Crick base pairs, non-canonical base pairs as demonstrated in Figure 4 can also be formed under appropriate conditions. The non-Watson–Crick interactions arise from the nucleotide bases having multiple hydrogen donor and acceptor sites. Typically, the amino group on A and C, O6 of G, and N7 of A or G that do not participate in the Watson–Crick base pairing can donate and accept hydrogen atoms, respectively. Moreover, protonation of N1 of A and N3 of C at acidic pH alters the hydrogen-bonding property, leading to a change of the base pair partner.[4]

An important alternative base pair in DNA is the Hoogsteen pair of T/A and C^+/G in a parallel-stranded orientation (Figures 4a and 4b). Thymine or protonated cytosine in the third strand embedded in the major groove of a DNA duplex forms two hydrogen bonds and adopts the base triplets of T/A/T and C^+/G/C, respectively (Figure 5a). The pH-dependent prediction parameters for a DNA triplex formation have been reported.[17] Another important non-canonical structure is the four-stranded helix by G-rich or C-rich nucleotides. The G-rich sequence forms a tetrad of hydrogen-bonded guanine bases by Hoogsteen-type hydrogen bonding (Figure 4c). The center of stacked G-tetrads surrounded by O6 of guanine can be a metal ion binding site and a G-quartet structure is formed (Figure 5b). Orientation of the DNA strands in the G-quartet structure is either antiparallel adopting a syn–anti alternation at the glycoside bond[18] or parallel with an anti configuration,[19] depending on the sequence and the metal ion included. The four-stranded structure formed by C-rich strands, called an *i*-motif, is composed of two parallel intercalated C/C^+ base pairs in which individual cytosine bases adopts an anti configuration (Figures 4d and 5c). Due to the complexity of their conformation and thermodynamic analysis, predictions of the four-stranded DNA structures and their thermodynamic stability are to date difficult. I will discuss later the importance of these structural diversities through non-Watson–Crick base pairs on constructing functional nanobiodevices and nanobiomaterials.

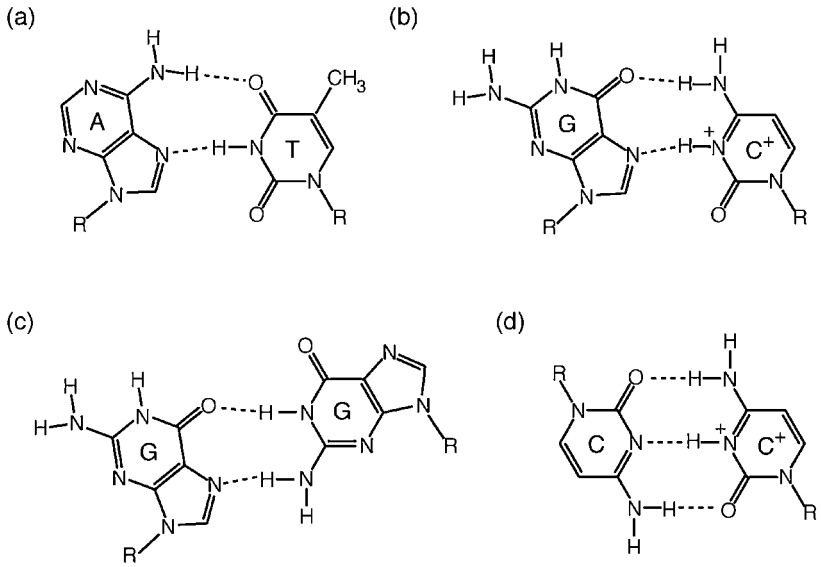


Figure 4. Non-canonical base pairs of (a) A/T, and (b) G/C$^+$, (c) G/G, and (d) C/C$^+$.

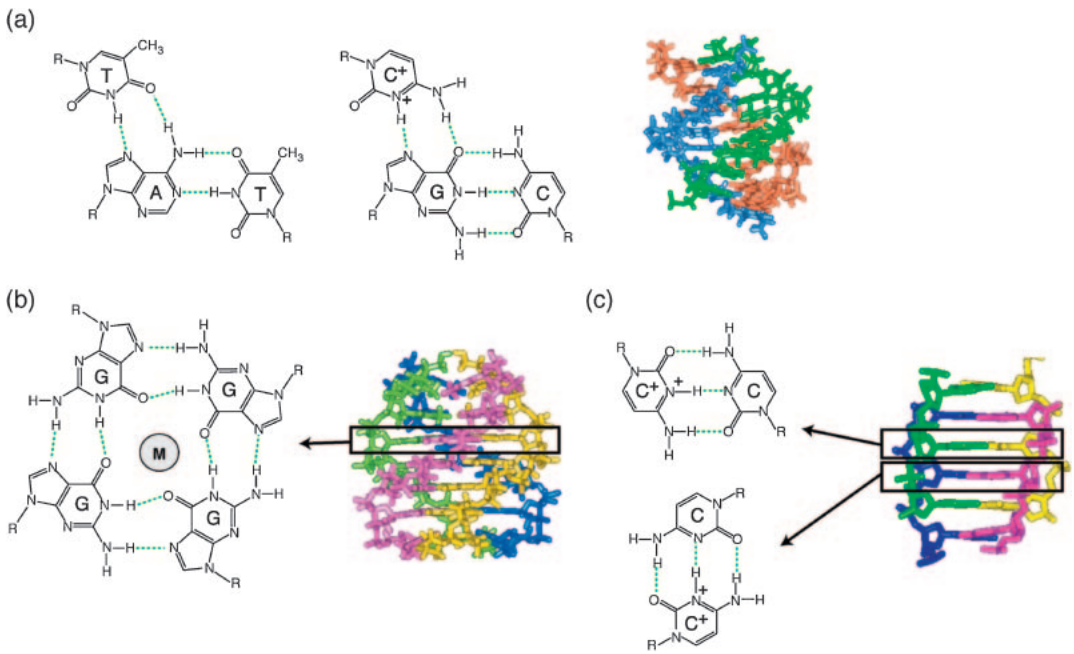


Figure 5. (a) Base triads of T/A/T and C$^+$/G/C. (b) Base tetrad of guanine bases coordinating metal ion (M). (c) Base pairs of C/C$^+$ forming two parallel duplexes intercalated into each other.

2. Extraction of "New Quality" from the Quantity of DNA

With the thermodynamic parameter of nucleotide interactions, it is now possible to predict secondary structures of DNA and RNA from their sequences.[7] The fundamental idea for the prediction is that the secondary structure which a nucleotide adopts is the most stable conformation thermodynamically. The power of the thermodynamic parameters has been estimated, for example, from the prediction of intracellular RNA structures, and it was found that the current algorithm using the nearest-neighbor parameters can predict approximately 80% and more RNA secondary structures found in a cell.[20,21] Therefore, the thermodynamic data for nucleotide interactions enable us to design DNA secondary structures and object-oriented nucleotide sequences, leading to an extraction of "new quantity" from the quality of DNA. In this section, I introduce two examples of our recent trials for the design of new functional DNA molecules based on the thermodynamic data of nucleotide interactions. First, base pair-mimic nucleosides are described. Since base stacking can provide interaction energy as much as the hydrogen bonds, artificial nucleosides tethering an aromatic hydrocarbon group were synthesized and incorporated in a DNA strand to expand this property. Second, a DNA switch by adding transition-metal ions is described. A transition metal-coordination group was incorporated in a DNA strand and the regulation of a DNA structure by transition-metal ion was examined. I show these artificial molecules exhibiting desired functions and describe the

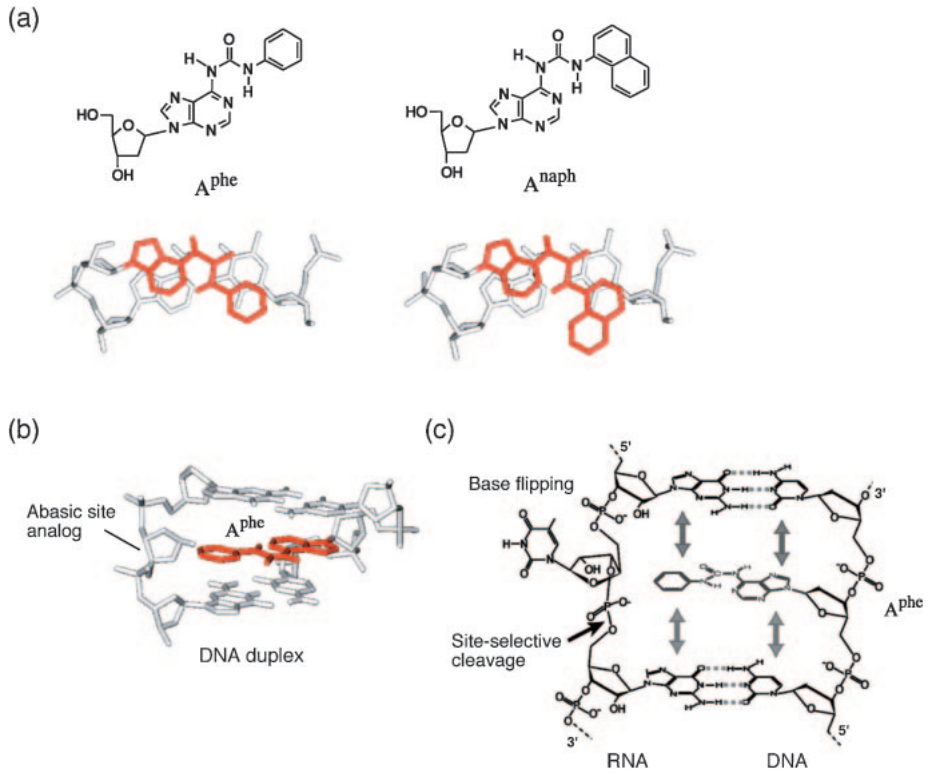


Figure 6. (a) Chemical structures of the deoxyadenosine derivatives of A^{phe} and A^{naph} as base pair-mimic nucleosides. Models of (b) the intercalation of the phenyl group of A^{phe} opposite the tetrahydrofuran abasic analog in a DNA duplex and (c) the A^{phe}-mediated RNA hydrolysis.

concept of an extraction of a new quality of the DNA from the data of nucleotide interactions.

2.1 Consideration of Nucleotide Interaction toward the Design of New Nanobiomolecules. 2.1.1 Design of Base Pair-Mimic Nucleosides: Based on the interaction energy data of Watson–Crick base pairs, a number of artificial nucleosides have been explored. Some are intended to enhance the ability of sequence recognition, in which artificial nucleosides changing the hydrogen-bonding patterns or the number of hydrogen bonds are examined.[22–24] Most other nucleotides are modified to have an expanded base size to enhance the stacking interaction. Planar aromatic molecules are often examined because the compounds have high potential for the stacking interaction and may intercalate between base pairs in a duplex.[25,26] Notably, it is reported that even a very large aromatic group such as pyrene[27] and porphyrin[28] increases the duplex stability.

In order to design artificial nucleosides that perturb a nucleotide duplex structure less significantly, we focused on the competitive relationship between the interaction energies of the hydrogen bond and the base stacking, indicated in Figure 2. According to the properties of nucleotide interactions, it is supposed that less hydrogen-bonding energy gives a greater stacking energy,[8] and extremely, no hydrogen bond might give the greatest base stacking. Based on the idea, we examined deoxyadenosine derivatives containing a phenyl group [N^6-(phenylcarbamoyl)-2′-deoxyadenosine, A^{phe}] and a naphthyl group [N^6-(naphthylcarbamoyl)-2′-deoxyadenosine, A^{naph}] at *N*6 of deoxyadenosine (Figure 6a), of which the synthesis and incorporation into a DNA strand were relatively simple. The phenyl group and the naphthyl group were examined to investigate significance of the stacking area on the stacking energy. Although the aromatic hydrocarbon groups are expected to stack in a DNA duplex when adopting base pair-mimic geometry, of which the aromatic group occupies the Watson–Crick face of deoxyadenosine, the deoxyadenosine derivatives also allow the base pairing with T or U when orienting the aromatic group out of the helix.

2.1.2 Strong Stacking by the Pair-Mimic Nucleosides: We investigated the thermal stability of the DNA duplexes containing the base pair-mimic nucleosides in 1 M (1 M = 1 mol dm^{-3}) NaCl–phosphate buffer at pH 7.0. To study the stacking of the deoxyadenosine derivatives, we evaluated the thermodynamics of DNA duplexes bearing the dangling A^{phe} or A^{naph}. We found that the single A^{phe} and A^{naph} at the 5′ dangling ends stabilized the DNA duplex by 1.7–0.9 kcal mol^{-1} per base pair-mimic nucleoside, which was much greater than that obtained by a natural adenine (0.5 kcal mol^{-1}).[29] Importantly, the stabilization energy by the dangling A^{phe} or A^{naph} was as much as or greater than that of the formation of A/T Watson–Crick base pairs (1.0–0.9 kcal mol^{-1}), which was contributed from the favorable entropy probably due to the desolvation from the aromatic groups. The large stabilization energy suggests that the aromatic groups efficiently overlap with the adjacent terminal base pair by adopting the Watson–Crick base pair-mimic geometry (Figure 6a). Notably, the stabilization energies of the DNA duplexes containing A^{phe} and A^{naph} were similar to each other, suggesting that even the phenyl group provided a maximized stacking energy and the overlap area was not the major determinant for the stacking energy. It is

interesting that the simple aromatic hydrocarbon groups of the phenyl group and the naphthyl group with no dipole or hydration site can provide interaction energy as much as natural DNA base pairs, while the natural nucleotide base stacking is mediated by the combination of electrostatic, hydrophobic, and dispersive forces.

Further study using 11-mer DNA duplexes containing A^{phe} or A^{naph} in the middle of the sequence showed that the overall structure of the B-form duplex was affected less by the incorporation of A^{phe}.[30] One of the difficulties in constructing appropriate experimental systems is how to minimize disruptions of the base pair-geometry, but A^{phe} and A^{naph} appear to overcome the problem because of their chemical structures analogous to the base pairing. It was a remarkable feature that ΔG°_{37} values for forming the DNA duplex containing A^{phe} or A^{naph} opposite any nucleotide species (A, G, C, and T) were similar to each other and the values were also similar in that case of using of a tetrahydrofuran abasic analog (Figure 6b). The observation suggests base flipping conformation opposite the base pair-mimic nucleoside by intercalating the aromatic hydrocarbon group into a DNA duplex. Although the base flipping involves a large energy penalty, it is probable that an intercalation of the phenyl and the naphthyl groups compensates the energy.

2.1.3 Site-Selective RNA Cleavage: The thermal stability of 11-mer RNA/DNA duplexes containing A^{phe} in the middle of the DNA sequence was also investigated using a 1 M NaCl–phosphate buffer at pH 7.0. The T_m (melting temperature) values among the natural duplexes containing A/A, A/G, A/C, and A/U pairs differed by 12.9 °C.[31] The large difference was due to the formation of A/U Watson–Crick base pairs and single mismatch pairs of A/A, A/G, and A/C in the middle of the RNA/DNA duplex. In contrast, the T_m values of duplexes containing A^{phe} in place of A to form A^{phe}/A, A^{phe}/G, A^{phe}/C, and A^{phe}/U pairs were almost the same (only 0.8 °C differences in their T_m values). This observation suggests that the aromatic hydrocarbon group of A^{phe} intercalveates into the duplex and the opposite nucleotide base is located in an unstacked position.

Site-selective RNA cleavage is important for biotechnology and therapeutics.[32] It has been indicated that the duplex backbone geometry prohibits the formation of the in-line attack arrangement while unpaired ribonucleotides can be preferentially hydrolyzed.[33,34] Thus, RNA hydrolysis is favored when the arrangement of the 2′-hydroxy group and the 5′-leaving oxygen atom adopts an in-line configuration during the transesterification reaction. It has been reported that an RNA or DNA strand associating with a cDNA which has an unnatural moiety that induces the base flipping of the opposite nucleotide can be hydrolyzed site-selectively.[35,36] Likewise, if A^{phe} induces the opposite nucleotide base flipping into an unstacked position, the ribonucleotide opposite A^{phe} may be hydrolyzed. To test this idea, the 11-mer RNA/DNA duplexes were examined for the RNA hydrolysis at 37 °C in the presence of divalent metal ions acting as a general base or acid. We observed that the RNA strand was efficiently hydrolyzed on the 3′-side of the ribonucleotide opposite A^{phe}.[31] Importantly, the RNA cleavage was highly site-selective, and any RNA sequence was cleaved when hybridizing with DNA containing A^{phe}. The RNA-hydrolyzing activity suggests that A^{phe} stacks into the duplex stably and rigidly and that the ribonucleotide opposite A^{phe} flips into an unstacked position regardless of the nucleotide species (Figure 6c). The site-specific RNA-cleaving activity of the base pair-mimic nucleoside verifies the base flipping model and suggests using the unnatural nucleoside as a catalytic core of a universal deoxyribozyme.

In conclusion from the base pair-mimic nucleoside studies, we found strong stacking interaction of the simple aromatic hydrocarbon group with minimized structural perturbation of the B-form structure while it forced an opposite nucleotide base flip out of the helix. The base flipping is important not only for RNA and DNA hydrolysis but also for DNA repair by repair proteins.[37] The ability of the base flipping agrees with the fact that the stacking interaction can be comparable to or even greater than the formations of the interbase hydrogen bonds. The strategy of such an artificial nucleoside design based on the property of nucleotide interactions would be powerful for DNA design with a desired function.

2.2 The Polymorphic Nature of Nucleic Acids toward New Nanobiodevices and Nanobiomaterials. 2.2.1 The DNA Structural Switch Induced by Surrounding Conditions: The structural conversions between duplex and noncanonical structures or among multiple noncanonical structures can be controlled by regulating their thermodynamics. These structural conversions regulated by various signals including the surrounding conditions are promising for the development of dynamic and switchable DNA nanodevices.

The G-quadruplex, which can be formed by intermolecular or intramolecular association of G-rich sequences in antiparallel or parallel orientations, shows a high structural polymorphism that depends on surrounding conditions (Figure 7a).[38] The G-rich sequences are observed in the whole genome, especially at the end of eukaryotic chromosomes, called the telomere region. We reported that only 1 mM of divalent cations largely affects the thermodynamic parameters of the antiparallel G-quadruplex of *Oxytricha* telomere DNA, $d(G_4T_4G_4)$[39] and more than 10 mM alkaline earth ions such as Ca^{2+} and Mg^{2+} can induce a structural transition from an antiparallel to a parallel G-quadruplex of $d(G_4T_4G_4)$.[40] G-quadruplexes are more polymorphic under molecular crowding conditions of neutral cosolutes. For example, molecular crowding of PEG causes $d(G_4T_4G_4)$ and $d(G_4T_4)_3G_4$ to undergo transitions from antiparallel to parallel G-quadruplexes (Figure 7b).[41] More drastically, our group found that a duplex formed with telomere DNAs composed of G-rich sequences and complementary C-rich sequences under dilute conditions dissociated upon molecular crowding and that the G- and C-rich sequences individually folded into quadruplexes (Figure 7c).[42] The duplex–quadruplex structural switches of telomere DNAs can be also induced by monovalent and divalent cations and pH.[43,44] These structural polymorphisms of DNA sequences are useful for the design of dynamic DNA devices as discussed in the next section.

2.2.2 DNA Logic Gates Based on Structural Polymorphisms: Molecular logic gates are key components of molecular devices such as molecular circuits, and they are the factor limiting the size of integrated circuits. Sequence-specific duplex formation of DNA through Watson–Crick base pairs

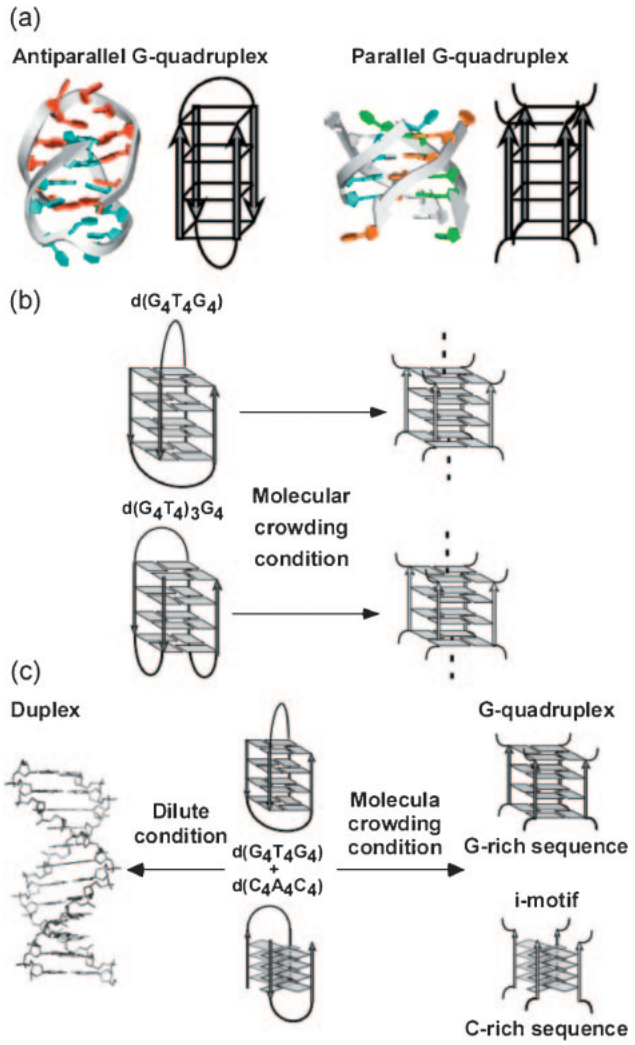
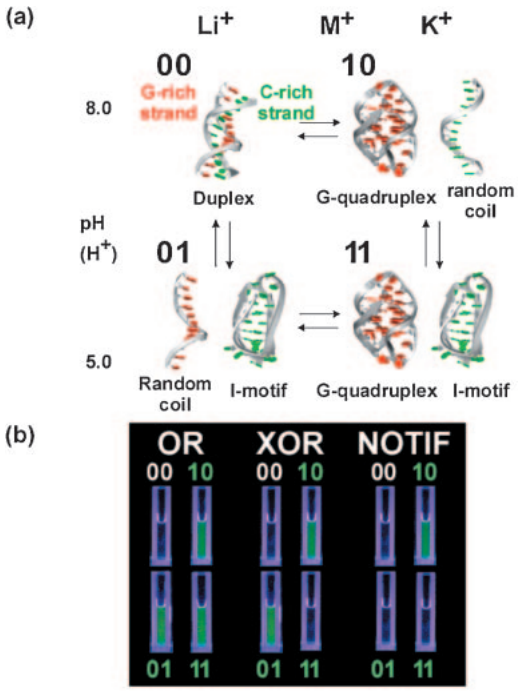


Figure 7. (a) Structures of an antiparallel G-quadruplex (left) and parallel G-quadruplex (right). (b) Schematic illustration of the structural transition of $d(G_4T_4G_4)$ and $d(G_4T_4)_3G_4$ from the antiparallel to parallel G-quadruplex induced by molecular crowding. (c) Schematic illustration of the duplex–quadruplex transition of telomere DNAs regulated by molecular crowding.



(c)

Input signal		Output signal						
i_1	i_2	AND	OR	NAND	NOR	XOR	XNOR	NOTIF
0	0	0	0	1	1	0	1	0
1	0	0	1	1	0	1	0	1
0	1	0	1	1	0	1	0	0
1	1	1	1	0	0	0	1	0

Figure 8. (a) Four structural states of telomere DNAs regulated by monovalent cation (M^+) and pH (H^+). (b) Logic operations of the telomere DNAs with fluorescent output signals. (c) Basic logic gates utilized in information processes which can be designed by use of telomere DNAs.

is appropriate for molecular devices. In fact, there have been many reports of DNA devices employing duplex formation.[45,46] However, the DNA duplex structure through Watson–Crick base pairs is not sensitive to the surrounding conditions. Therefore, DNA duplexes are not sufficient for constructing molecular logic gates, wherein the molecules must respond to signals such as the surrounding conditions. Complex combinations of sequence-specific duplex formation of DNA and functional molecules including enzymes and deoxyribozymes have been employed for the development of DNA-based logic gates.[47] High-ordered DNA structures, which consist of not only Watson–Crick base pairs but also non-Watson–Crick base pairs, have even more useful properties for simple design of logic gates, because their structure and stability can be controlled by surrounding conditions without the addition of complex devices. The development of simple and designable molecular logic gates without the need for complex devices should stimulate further studies on molecular circuits.

From these points of view, we studied systematically the structure and stability of telomere DNAs and found that the dynamic structural conversion of telomere DNAs among G-quadruplex, i-motif, random coil, and duplex forms was controllable by both monovalent cations and pH (Figure 8a).[48] Based on this drastic structural conversion, we propose a new concept for the development of a molecular logic gate. As a proof-of-concept, we demonstrate that telomere DNAs can perform OR, XOR, and NOTIF logic gate functions in response to input signals (monovalent cations and pH), with fluorescence intensity changes as output signal (Figure 8b). Moreover, combinations of the structural polymorphism of telomere DNAs and fluorescence probes allowed us to design various DNA logic gates shown in Figure 8c. Although further studies for DNA logic gate operations are required, these results indicate that simple, rationally designed, and chemically synthesized biological molecules will promote further development of complicated and combinational molecular logic circuits. Especially for practical use, logic gate operations with DNA devices should be less power consuming and have lower

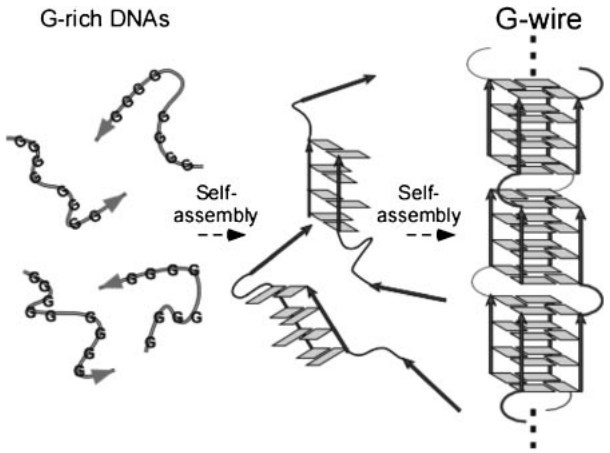


Figure 9. Schematic illustration of G-wire formation with numerous parallel-stranded G-rich DNAs. Gray square indicates guanine bases. Main chain of DNAs are omitted for clarity.

volatility than devices constructed of organic compounds.[49,50]

2.2.3 Dynamic DNA Nanostructure Controlled by Chemical Stimuli: The structural polymorphism of nucleic acids is the most promising property for development not only of dynamic nanobiodevices such as logic gates but also dynamic nanobiomaterials such as high-order DNA nanostructure based on G-quadruplexes called G-wire. G-wires consist of numerous strands of guanine-rich DNA strands aligned in parallel (Figure 9).

In order to induce G-wire formation by chemical stimuli, we investigated how surrounding conditions affect G-quadruplex structure. It was found that Ca^{2+} and molecular crowding induced G-wire formation of $d(G_4T_4G_4)$.[40,41] We further demonstrated that a single G to A substitution in the loops leads to a drastically different structure under molecular crowding conditions. Under crowding conditions, $d(T_2(G_3T_2G)_3G)$ folds into very long G-wires, whereas $d((G_3T_2A)_3G_3)$ folds into antiparallel G-quadruplexes.[51] Besides the biological aspects of the G-rich sequences such as telomere DNA,[52] there is growing interest in G-rich sequences as functional elements in molecular electronics.[53] A theoretical study suggested that the G-wire structure was promising for nanoscale biomolecular electronics because of its high-order structure.[54] However, its electrical properties have not been described yet because of the difficulty in creating and controlling the structure. Therefore, it is necessary to develop methods for controlling G-wire formation by manipulating the surrounding conditions. In addition, the regulation of formation should be useful to study oxidative damage by hole and electron transfer[55] which may be resolved by excess electron transfers or metallizations with DNA scaffolds.[56]

2.2.4 Artificial G-Wire Switch with 2,2′-Bipyridine Units: In order to develop controllable G-wire, it is necessary to design and synthesize a DNA that can undergo a reversible structural transition between a compact antiparallel G-quadruplex and a long G-wire depending on external signals. To enable the reversible structural transition, we replaced the thymidines in a chain of $d(G_4T_4G_4)$ with a metal ion-responsive unit, 2,2′-bipyridine, which is composed of two aromatic rings

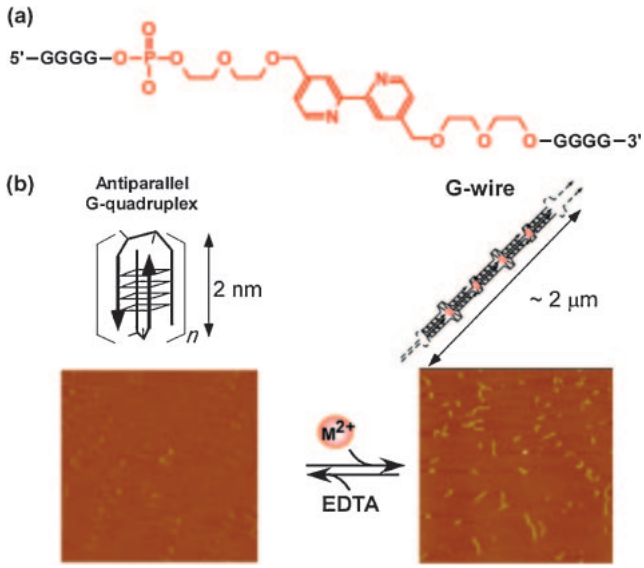


Figure 10. (a) Molecular design of artificial DNA containing a bipyridine unit instead of four thymines. The bipyridine unit is highlighted by red color. (b) Schematic structures of the artificial DNA in the absence (left) or presence (right) of metal ions and their AFM images. A red circle indicates metal ion.

that rotate arbitrarily upon coordination with metal ions (Figure 10a).[57] Structural analysis showed that the DNA containing the 2,2′-bipyridine units underwent a structural transition from an antiparallel G-quadruplex to a high-order parallel-stranded G-quadruplex upon addition of divalent metal ions such as Ni^{2+}, Co^{2+}, and Zn^{2+}. In addition, Na_2EDTA induced a reversible structural transition from the high-order structure to the antiparallel G-quadruplex. Moreover, direct observation of the reversible structural transition by AFM (atomic force microscope) shows that the high-order parallel-stranded structure is a G-wire that contains numerous DNA oligonucleotides, which demonstrates that the G-wire can be switched by divalent metal ions and Na_2EDTA (Figure 10b). Alternating additions of Ni^{2+} and Na_2EDTA showed that the G-wire could be switched up to about 10 times, with a cycling efficiency of 0.95 (i.e., only 5% loss per cycle). These results demonstrate that the G-wire switch developed here can be readily controlled by chemical signals. Notably, the G-wire switch that we developed has two important properties which are useful for the development of DNA-based functional nanomaterials. First, incorporation of bipyridine units into G-quadruplexes allowed design and synthesis of a self-assembled G-wire switch that could be controlled by external stimuli, namely divalent metal ions and a chelating agent. Second, when the G-wire switch developed here binds divalent metal ions, it creates a one-dimensional array of divalent metal ions that is much longer than that reported previously. We have reported that telomere DNAs behave as molecular logic gates that respond to chemical input signals as shown above. Our results indicate that it should be possible to produce artificial and natural telomere DNAs that can regulated by the surrounding conditions for use as novel functional nanobiomaterials that are switchable, controllable, and addressable.

3. Conclusion and Perspectives

Nucleotide interaction is governed by the thermodynamics in which nucleotides form a structure with the lowest free energy. Studies of the thermodynamic data have revealed the principles of nucleotide interactions of hydrogen bonding and base stacking. The approach from thermodynamics also enables prediction of the secondary structure of an oligonucleotide and its thermal stability, thus the design of a functional oligonucleotide sequence becomes easier. The advantages of oligonucleotides raise the idea that DNA is one of the most promising molecules as a functional nanobiodevice and nanobiomaterial. Since there are limited kinds of nucleotides in nature, development of new built-up nucleotide units is quite important. Understanding nucleotide interaction energies is essential to design functional nucleotide sequences and artificial nucleosides. The quantity data obtained with artificial DNA molecules can reinforce the quantity data of nucleotide interactions.

Extensive studies of the thermodynamics of DNA and RNA structures have enabled us to design nucleotide sequences. However, most thermodynamic data were obtained from in vitro evaluations using a homogeneous aqueous solution, although reactions occurring in living cells are usually much more effective and rapid compared to those observed in vitro. Furthermore, many molecular properties that are not observed in vitro may appear as a result of the intracellular environment, referred to as the molecular crowding that modulates molecular conformation, molecular activity, and the properties of solvent water.[58] Accordingly, studies of nucleotide properties under molecular crowding conditions are also important for nucleotide designs that work efficiently in living cells or activate after enduring intracellular conditions.[59–62] The ability of DNA to respond to molecular environment can also be used for monitoring a target molecule.[53–66] It is also important to study the dynamics and the kinetics of nucleotide interactions. However, there has been no systematic study of them, so the prediction of nucleotide properties regarding reaction kinetics is impossible at present. If we could design and control functional DNAs, biotechnology and nanotechnology utilizing DNA would expand application fields. Design of DNA functions both from the viewpoints of thermodynamics and kinetics would open a new field of nanobiotechnology.

I am grateful to the colleagues named in the cited papers released from our laboratory, especially Drs. S. Nakano and D. Miyoshi. I am also grateful to the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan and Konan Gakuen for support of this work through Grants-in-Aid for Scientific Research, "High-technology Research Center" Project (1999–2004), "Academic Frontier" Project (2004–2009), and "Hirao Taro Foundation of the Konan University Association for Academic Research" Project (2008–2013). I am further grateful to Hyogo Science and Technology Association for the financial support.

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Naoki Sugimoto received Ph.D. in 1985 from Kyoto University, Japan. After postdoctoral work at University of Rochester in USA, he joined Konan University, Kobe, Japan in 1988 and is a full professor since 1994. From 2003, he holds a director of Frontier Institute for Biomolecular Engineering Research (FIBER) at Konan University. He is a committee member of Hyogo Science and Technology Conference from 2005, and a member of the Editorial Board of the Nucleic Acids Research from 2007. He received the Dr. Masao Horiba's Award in 2004, Distinguished Scientist Award from ICA (International Copper Association), New York, USA in 2005, Hyogo Science Award from Hyogo Prefecture, JAPAN in 2006, the CSJ Award for Creative Work in 2007, and so on. His research interests focus on biophysical chemistry, biomaterials, nanobio-engineering, molecular design, biofunctional chemistry, biotechnology, etc. Prof. Sugimoto has published more than 400 scientific papers and books.