DNA Nanotechnology

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DNA–Metal Base Pairs

*Guido H. Clever, Corinna Kaul, and Thomas Carell**

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Recent developments show encouraging results for the use of DNA as a construction material for nanometer-sized objects. Today, however, DNA-based molecular nanoarchitectures are constructed with mainly unmodified or at best end-modified oligonucleotides, thus shifting the development of functionalized DNA structures into the limelight. One of most recent developments in this direction is the substitution of the canonical Watson–Crick base pairs by metal complexes. In this way "metal–base pairs" are created, which could potentially impart magnetic or conductive properties to DNA-based nanostructures. This review summarizes research which started almost 45 years ago with the investigation of how metal ions interact with unmodified DNA and which recently culminated in the development of artificial ligand-like nucleobases so far able to coordinate up to ten metal ions inside a single DNA duplex in a programmable fashion.

1. Introduction

Sophisticated molecular nanotechnology will require the hierarchical self-assembly of discrete functional molecular entities into complex nanoarchitectures.[1] The vision is that future "nanoconstruction plans" may allow the creation of functional molecular machines and self-organizing nanoobjects with interesting physical properties.[1,2] Towards this goal, chemists are starting to use biological molecules, which frequently possess superior properties for self-assembly, optimized through billions of years of evolution. Within this context, the use of DNA for the creation of nanoobjects is currently of great interest.[3] The advantage is that today the synthesis of small oligonucleotides is chemically trivial. In addition, we know how to assemble oligonucleotides into defined double, triple, or quadruple strands, into branched structures or hairpins. We also understand increasingly how these DNA structures can be used to assemble large nanoarchitectures.[4] Longer DNA strands carrying modifications can be prepared using the methods of ligation[5] or the polymerase chain reaction (PCR),[6] and all these oligonucleotides are amenable to further enzymatic modification using the plethora of enzymes provided by molecular biologists.[7]

Fascinating examples of static three-dimensional architectures such as Seeman's cube have been constructed using DNA or RNA strands. Other structures have been created using unmodified oligonucleotides or even DNA strands carrying junction nodes or unnatural functional groups at their ends.[4,8,9] The DNA-based programming of the self-assembly of hundreds of well-chosen oligonucleotides recently furnished amazingly complex surface structures (such as "smileys"), which were visible by AFM.[10] Several groups reported the use of DNA sequences for the generation of surface-bound lattices or soluble nanoobjects able to bind proteins, organic molecules, or inorganic nanoparticles.[3,11,12] Furthermore, several molecular machines that exhibit controlled motion on nanometer dimensions were constructed from oligonucleotides.[13,14]

Metals are in many respects the carriers of functions particularly desired in the nanoworld.[1,15] Nanoscopic metal wires may allow conducting electrons through self-assembled networks. Magnetic coupling of the metal ions may create nanomagnets with defined orientation and strength of the magnetic fields. The combination of the biomolecule DNA, with its superior self-assembly properties, and functional building blocks such as metal ions is therefore one of the most promising avenues towards the goal of constructing complex functional nanoarchitectures.

The covalent attachment of metal complexes to oligonucleotides has been reported in various contexts such as energy transfer[16] or electron transfer through DNA,[17] construction of synthetic endonucleases,[18] and as end-bound nodes for the

[*] Dr. G. H. Clever, C. Kaul, Prof. Dr. T. Carell
Department of Chemistry and Biochemistry
Ludwig Maximilians University Munich
Butenandtstrasse 5-13
Haus F, 81377 Munich (Germany)
Fax: (+49) 89-2180-77756
E-mail: Thomas.Carell@cup.uni-muenchen.de

assembly of artificial DNA constructs.[19] One example was reported by Han et al., who assembled complex nanostructures from DNA single strands end-modified with terpyridines by means of stable bis(terpyridine)iron(II) complexes.[20] Star-shaped constructs with several oligonucleotide "rays" protruding from a central Ni^{II}–cyclam or Ru^{II}–tris(bipyridyl) complex were reported by Steward and McLaughlin.[21,22] A different strategy, developed by Sheppard et al., used the sequence-specific formation of DNA double strands to template the formation of metal complexes between ligands bound to the terminal phosphates of two single strands bound to the template.[23,24] Gothelf et al. equipped linear or tripodal nanoscale building blocks with oligonucleotides as recognition sequences for the specific build-up of large aggregates, which were finally connected by metal complexes.[25] The decoration of oligonucleotides with metal complexes bound to the DNA backbone through alkyne linkers was shown to be possible during solid-phase synthesis by Tor et al. using a modified phosphoramidite bearing a ruthenium (or osmium) phenanthroline complex.[26] Sleiman et al. reported on the synthesis of a branched Ru^{II}-DNA complex, in which two parallel DNA strands were linked together.[27,28]

When the hydrogen-bonded Watson–Crick base pairs are replaced by metal–ligand interactions inside the DNA double helix, a "metal–base pair" is formed. Certain metal ions can be coordinated either by a pair of natural nucleobases or by specially designed ligand nucleosides, which are placed opposite each other in the double helix. Herein, we describe the currently known metal–base pairs with respect to the type of ligand used, number and identity of coordinated metals, and the properties of the resulting double strands.[29] Examples of metal-stacking and metal-mixing inside the DNA double helix are given.

Guido Clever studied chemistry at the University of Heidelberg. He joined the Carell group in 2003 and worked on the development of the metal–salen base pair for which he was awarded the Klaus Römer award. In 2006 he obtained his PhD from the Ludwig-Maximilians-University Munich. He was fellow of the Studienstiftung d. dt. Volkes and a Kekulé fellow of the FCI. Since 2007, he has been a postdoctoral JSPS fellow in the group of group of Prof. Shionoya at the University of Tokyo.

Corinna Kaul received her BSc (2004) in chemistry and biochemistry and her MSc (2006) in chemistry from the Ludwig-Maximilians-University Munich. In 2006 she started her PhD studies under the supervision of Prof. Carell studying metal-mediated base pairs.

Thomas Carell obtained his PhD from the University of Heidelberg in 1993 (Prof. Staab). After postdoctoral research at MIT with Prof. Rebek, he moved to the ETH Zürich as an independent group leader associated with Prof. Diederich. In 2000 he became full professor for Organic Chemistry at the Philipps-University in Marburg, and in 2004 he moved to the Ludwig-Maximilians-University Munich. His awards include the Leibniz Award (2004) and the Philip Morris Research Award (2006).

2. Coordination of Metals to Unmodified DNA

The interaction of metal salts with unmodified DNA was examined long before the secondary structure of the DNA duplex had been elucidated.[30] Complexes of metal ions with DNA were later named M-DNA by Lee et al.[31] The research field of metal-complexing DNA can be divided into the following topics: 1) the formation of non-canonical base pairs from the natural nucleobases with participation of metal ions;[31] 2) the exchange of hydrogen atoms that are part of the Watson–Crick base pairing by metal ions;[31] 3) the reversible binding of metal ions to parts of the DNA not involved in base pairing;[32,33] and 4) the persistent distortion or crosslinking of DNA duplexes by metal ions (mainly platinum) that form kinetically inert complexes.[34] Only examples of the first two classes will be discussed here. In addition it is noteworthy that new insights into the binding of Mg^{2+} ions to the catalytic RNA duplexes of group-II-intron ribozymes were recently reported by Sigel et al.[35] For information on intercalation of metal complexes into the base stack we refer the reader to an excellent review.[36]

2.1. Thymine–Thymine Mismatches Coordinating Hg^{2+}

In 1952 Katz found a substantial decrease of the viscosity of natural DNA upon addition of $HgCl_2$, which he attributed initially to a decrease of the overall size of the molecule.[37] First he believed that mercury ions bind to the phosphate groups in an intrastrand fashion. But after Thomas proved binding of Hg^{2+} to the nucleobases by UV spectroscopy,[38] Katz proposed in 1963 the formation of Hg^{2+}–thymine (1:2) complexes in DNA double strands. He postulated a slippage process which was thought to bring two thymine bases in the two strands together to form a Hg^{2+}-connected metal–base pair.[39] This proposed structure for the T-Hg-T base pair **1** was depicted in his original paper and was later shown to be correct (Figure 1).

A crystal structure of 1-methylthymine complexing Hg^{2+} (2:1) further supported this idea.[40] Binding studies showed that the strength of the Hg^{2+} interaction increased with arising AT content.[41] Gruenwedel studied the interaction of mercury(II) ions with DNA strands using UV and CD spectroscopy and observed major transitions in the secondary structure upon Hg^{2+} binding, indicating that DNA moves from the canonical B-duplex structure upon binding of Hg^{2+} to a new, yet unknown, structure.[42]

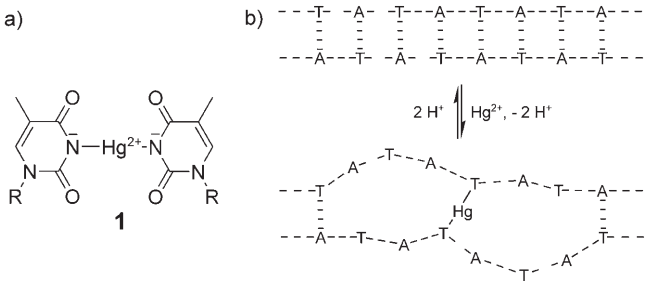


Figure 1. a) The structure of the T-Hg-T base pair **1** suggested by Katz in 1963. b) His proposed model for the formation of T-Hg-T base pairs by a chain-slippage process.

This metal–base pair concept was further elaborated by Buncel et al. and Marzilli et al., who verified the formation of T-Hg-T inter- and intrastrand crosslinks in double strands containing one or more TT mismatches by UV and CD spectroscopic titrations and by NMR spectroscopy.[43,44] The Marzilli group reported the stacking of three Hg^{2+} ions inside a DNA duplex in 1996.[44] However, they observed that the stacking of more than three mercury ions was virtually impossible owing to an intrastrand hairpin formation process which was favored over duplex formation. Further support for the formation of the T-Hg-T base pair was reported most recently by Ono et al. who provided melting-curve studies and ESI mass spectrometric data proving the presence of Hg ions inside the DNA.[45,46] In a series of NMR experiments, in which they used DNA duplexes containing TT mismatches with $^{15}N(3)$-labeled T bases, the Ono group was able to verify the structure of the T-Hg-T base pair **1** on the basis of the $^2J_{NN}$ coupling constant.[47]

Ono et al. provided in addition direct evidence for the formation of a stack of three T-Hg-T base pairs in a small trimer duplex constructed from 5′-d(T_3)-3′ (Figure 2). The

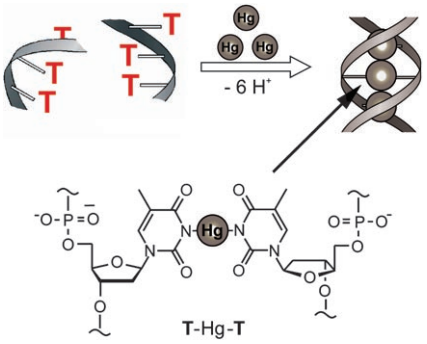


Figure 2. Formation of three T-Hg-T base pairs from the simple trinucleotide 5′-d(T_3)-3′ upon addition of Hg^{2+} ions.[46]

group also provided evidence by ESI mass spectrometry for the stacking of up to five Hg^{2+} ions inside a DNA duplex.[46] This data showed that Hg^{2+} ions and dT-only oligonucleotides form complexes, in which two opposite thymines can efficiently assemble to the T-Hg-T base pair upon addition of Hg^{2+}.

2.2. Exchange of H Atoms in Base Pairs by Metal Ions

Various reports describe complexes of unmodified DNA double strands with divalent metal cations. NMR- and titration-based analyses of the imino proton signals of the N(3)-H atom of thymine or the N(1)-H atom of guanine by Zn^{2+}, Co^{2+}, and Ni^{2+} ions (at high pH) conducted by Lee et al. provided data that fit the structural models depicted in Figure 3 for the complexes formed.[48,49]

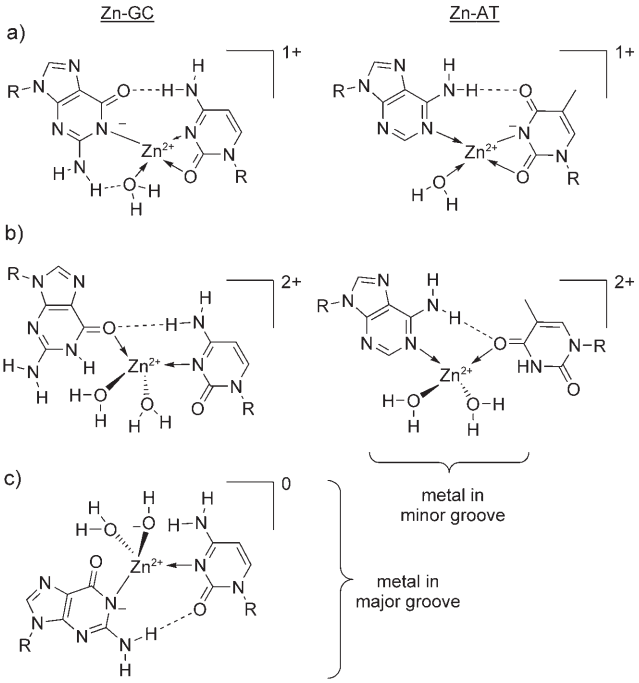


Figure 3. Proposed structure of the Zn^{2+}-coordinated AT and GC complexes formed in DNA according to a) Lee,[48] b) Lippert,[56] and c) Alexandre.[57]

In Lee's model one proton is released for every bound divalent metal, depositing one net positive charge on the helix. Indeed, the positively charged intercalator ethidium bromide fails to interact with the metal-complexed DNA, which can be explained by charge repulsion. The absorption and circular dichroism spectra of the complexed DNA are very similar to those of B-DNA, making structural changes rather likely for the failure of ethidium bromide to intercalate. Lee provided data that suggest unusual electronic properties of the complexed DNA, indicating that the material may behave as a molecular wire, for example, for energy transfer.[50] The electron conductance of a 15-μm-long M-DNA strand was measured between two gold electrodes, and a metal-like conductance was observed in contrast to native B-DNA.[51] Measurements of B-DNA showed here that the conductance is rather limited[17,52,53] and may proceed by charge hopping.[54] The exact structures and electronic properties of the formed metal–DNA complexes are, however, still the subject of controversy,[55] and other possible structures were proposed by Lippert et al.[56] and Alexandre

et al.[57] (Figure 3). These and other related models were recently studied in more detail by Fuentes-Cabrera et al. using DFT methods.[58] Despite these theoretical models, however, a final conclusive description of which structures are finally formed could not be given; crystallographic investigations are now required.

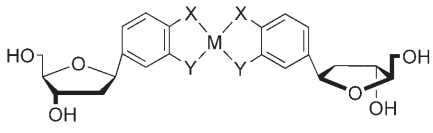
3. The Metal–Base Pair Concept

3.1. General Considerations

In the metal–base pair concept, coordinative forces are used to form a DNA duplex from two single strands. The Watson–Crick hydrogen bonds are replaced by the interaction between metal ions and the nucleosides acting as ligands. Three main coordination geometries are in principle possible for the arrangement of two bidentate ligand nucleosides around a central metal ion: square-planar, $D_2{}^d$, and tetrahedral. For maintaining the B-DNA-like structure for which π stacking is of paramount importance, the optimal coordination geometry of a metal–base pair should be square-planar or at most $D_2{}^d$. When metal ions that favor additional axial ligands in their coordination spheres are incorporated, their needs can be satisfied with loosely bound solvent molecules or bridging donor atoms from the neighboring bases. Metals that require a tight binding of apical ligands perpendicular to the base-pair plane are anticipated to cause major distortions of the double-helical structure (if they are coordinated at all).

3.2. First-Generation Metal–Base Pairs

The first example of an artificial ligand potentially suitable for the coordination of metal ions inside a DNA double helix was reported by Tanaka and Shionoya in 1999.[59] They synthesized the *o*-phenylenediamine–palladium complex **2** and later the derivatives **3**,[60] **4**,[61] and **5**[62] in solution but did not initially report the incorporation of these nucleosides into oligonucleotides (Figure 4).



2: X = Y = NH_2, M = Pd^{2+}
3: X = O^-, Y = NH_2, M = Pd^{2+}
4: X = Y = O^-, M = Cu^{2+}
5: X = Y = O^-, M = B^{3+}

Figure 4. The monomeric metal–base pairs **2**–**5** prepared by Tanaka and Shionoya.[59–62]

The first successful formation of a metal–base pair based on synthetic ligands instead of natural bases inside a DNA duplex was reported in 2000 by Meggers, Romesberg, and Schultz.[63] A combination of a pyridine-2,6-dicarboxylate (Dipic) as a planar tridentate ligand and a pyridine nucleoside (Py) was incorporated opposite each other in two complementary oligonucleotide strands. The addition of Cu^{2+} resulted in formation of the copper–base pair Dipic-Py **6**, which significantly stabilized the DNA duplex (Figure 5). Other metal salts such as $CeCl_3$, $Mn(NO_3)_2$, $Fe(SO_4)_2$, $Co(NO_3)_2$, $Ni(NO_3)_2$, $Zn(NO_3)_2$, $Pd(NO_3)_2$, and K_2PtCl_4 gave, however, no duplex stabilization.

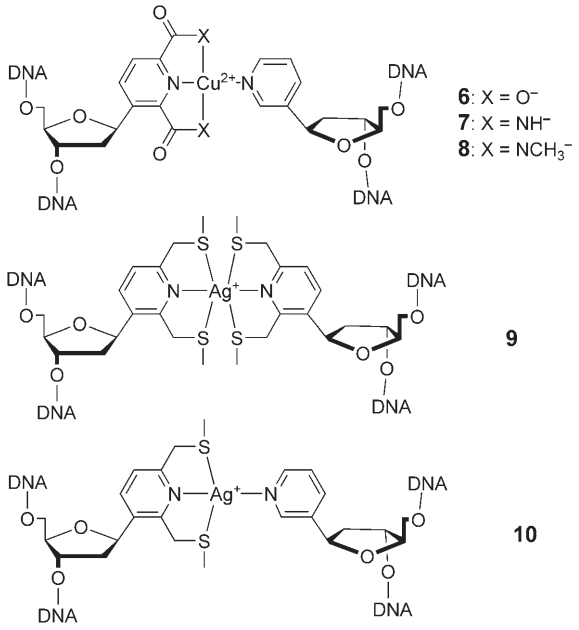


Figure 5. The metal–base pairs Dipic-Py **6**, Dipam-Py **7**, MeDipam-Py **8**, SPy-SPy **9**, and Spy-Py **10** from the Schultz group.[63–65]

The tridentate character of the pyridine-2,6-dicarboxylate moiety is primarily responsible for the tight binding to Cu^{2+} (whereas Zn^{2+}, Ni^{2+}, Pd^{2+}, and Pt^{2+} are only loosely bound). This ligand subsequently allows the coordination of the oppositely arranged pyridine nucleobase to the copper atom's fourth coordination site. The beauty of the concept of using such an unsymmetrical (3+1) arrangement is that a novel asymmetric metal–base pairing system, orthogonal to the Watson–Crick base pairs is created, which may also allow replication by DNA polymerases. This idea, however, still awaits realization.

The derivative Dipam-Py **7** leads to an even higher duplex stabilization than its predecessor (in contrast to the combination MeDipam-Py **8**, which does not form a stable metal–base pair at all).[64] The combinations SPy-SPy **9** and SPy-Py **10**, which selectively bind Ag^+ ions, were subsequently investigated and incorporated into oligonucleotides (Figure 5).[65]

The Schultz group was also able to incorporate two metal–base pairs **6** into the palindromic Dickerson–Drew[66] dodecamer to obtain the first crystal structure of a metal–base pair inside the DNA duplex. The whole DNA was found to exist in a Z-DNA-like conformation (Figure 6),[67] which is believed to be induced by the special sequence used for the experiment. Solution studies with other DNA sequences containing

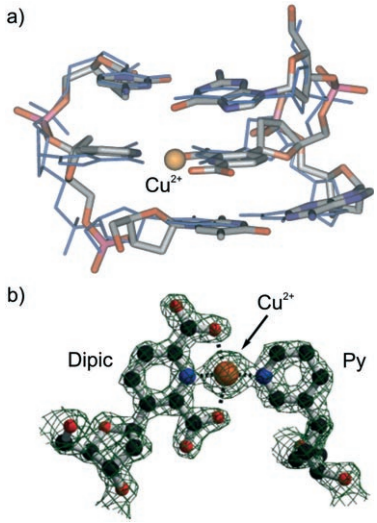


Figure 6. a) Local environment of one of the two Cu^{II} ions in the X-ray structure (1.5-Å resolution) of the duplex d(5′-CGCG*Dipic*AT*Py*CGCG-3′)$_2$ (thick rods; superposition with ideal Z_I-DNA, drawn in fine blue lines). b) Electron density of the Dipic-Cu^{2+}-Py base pair, contoured at 1.3 σ.[67] Reproduced with permission ©2001 American Chemical Society.

two metal–base pairs **6** indeed suggest a preferred B conformation in solution.[67]

Several other metal–base pairs were subsequently reported (Figure 7). Shionoya et al. prepared a pyridine nucleoside Py (**11**, Figure 7) for the formation of double and triple helices coordinating central Ag^+ ions.[68] This, however, seems to depend on the DNA sequence, because in other studies the Py-Ag^+-Py base pair did not form.[65] Shionoya and Tanaka also prepared the hydroxypyridone (H) base pair **12**, which was able to bind Cu^{2+} ions inside DNA double strands.[69] The

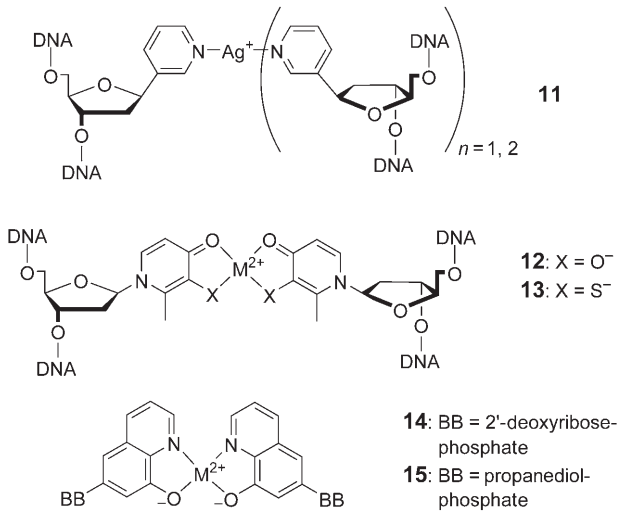


Figure 7. Depiction of Shionoya's $Ag^+(Py)_3$ base trio **11**,[68] his hydroxypyridone metal–base pair **12**,[69] and the tentative sulfur analogue **13**.[70] The hydroxyquinoline base pair **14** with a deoxyribose backbone and **15** with a simplified propanediol backbone were synthesized by the Meggers group.[71]

synthesis of the corresponding 3-sulfur-substituted analogue **13** is currently ongoing with the intention to allow the coordination of soft metals such as gold or palladium.[70] Using a 8-hydroxyquinoline (HQ) ligand, Meggers et al. was able to create the 2′-deoxyribosyl-based metal–base pair dHQ-dHQ **14** and in addition the propylene glycol backbone analogue pHQ-pHQ **15**.[71] Noteworthy is the fact that the metal–base pair concept was recently shown to work in PNA duplexes as well by Achim et al. (not shown).[72]

3.3. Metal Stacking with the Hydroxypyridone Base Pair

Recently, five consecutive copper–hydroxypyridone base pairs **12** were incorporated into a double strand by the Shionoya group (Figure 8). This DNA duplex was able to

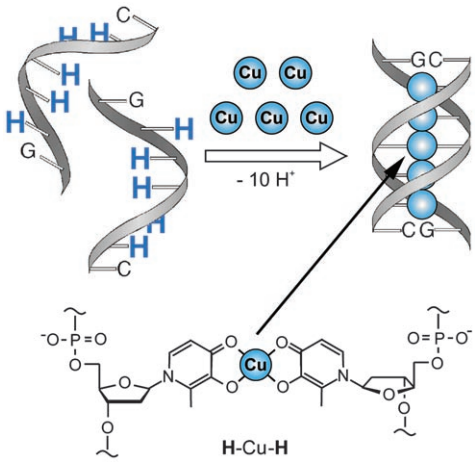


Figure 8. Schematic depiction of the assembly of a duplex containing five stacked Cu^{2+} ions consisting of a short palindromic oligonucleotide containing five consecutive hydroxypyridone ligands flanked by only one natural nucleobase on both ends.[73]

complex five Cu^{2+} ions in the center of the helix, which are supposed to stack on top of each other. These new materials were characterized by UV and CD titration experiments, EPR spectroscopy, and ESI mass spectrometry.[73] The EPR spectra indicated that the electron spins of the adjacent Cu^{2+} centers are aligned in parallel. A ferromagnetic coupling was observed with a spin state of 5/2 for the total system. Based on the EPR data, the distance between the copper centers was estimated to be 3.7 ± 0.1 Å, which is slightly larger than the analogous distance in natural B-type DNA (3.3–3.4 Å).

The theoretical investigations of this system by Di Felice et al. showed that the total magnetization of this ferromagnetic wire could depend linearly on the number of planes in the stack.[74] The formation of a high spin state was in accordance with the EPR data by Shionoya, supported by the calculation. The authors suggest that the combination of interplane spin coupling and intraplane metal–hydroxypyridone coupling is the important factor that determines the coupling. Although the nature of the σ and π frontier orbitals, with nodes between the stacked planes, does not support

bandlike electron conduction, it was anticipated that the efficient interaction of the metal–ligand systems in the duplex may support alternative conductance mechanisms driven by potential redox activities of the inner cations.

3.4. 2,2′-Bipyridine-Derived Metal–Base Pairs

Tor et al. coupled a 2,2′-bipyridine (Bipy) ligand by means of a methylene spacer to 2′-deoxyribose and obtained the metal–base pair **16**, which displayed a small duplex stabilization after addition of Cu^{2+} (Figure 9).[75] The geometry of this metal–base pair, however, does not allow a smooth fit into the double-helical structure. A related nucleoside carrying a bipyridyl unit directly connected to the sugar C1′ atom (i.e. **16** without the methylene groups) was investigated by Leumann et al. for ist effect on duplex stability in the absence and presence of transition-metal ions. Whereas duplex stabilization by two opposing bipyridine bases, in the absence of any metal ions, could be proven,[76] the influence of transition-metal ions remained unclear.[77] The two metal–base pairs PyA-PyA **17** and PyC-PyC **18**, derivatives of the natural nucleobases adenine and cytidine, were prepared by the Switzer laboratory (Figure 9).[78,79] Both metal–base pairs **17** and **18** showed an amazingly strong preference for the binding of Ni^{2+} relative to other transition-metal ions such as Co^{2+}, Cu^{2+}, Zn^{2+}, Fe^{2+}, and Mn^{2+} inside the duplex.

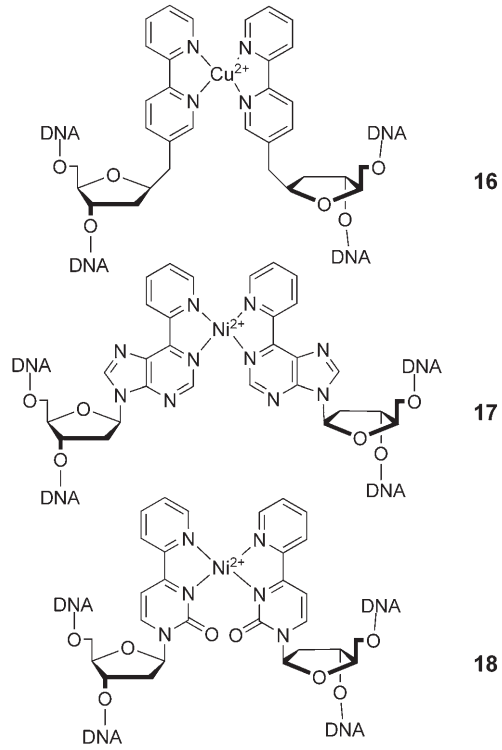


Figure 9. The 2,2′-bipyridyl metal–base pair **16** was developed by Tor et al.[75] Structurally related base pairs **17** and **18** were synthesized by Switzer et al. starting from the natural nucleobases adenine and cytidine by attachment of 2-pyridyl moieties by means of a transition-metal-catalyzed cross-coupling reaction.[78,79]

3.5. The Metal–Salen Base Pair: Combining Covalent Bridging with Metal Complexation

A completely different metal–base pair concept was developed based on the well-known *N*,*N*′-bis(salicylidene) ethylenediamine (salen) ligand attached to ribose as a C-nucleoside based on chemistry developed by the Seitz laboratory[80b] (Figure 10). The reasons for this choice were: 1) the high geometrical match between the flat metal–salen complex and the natural Watson–Crick base pairs and 2) the idea that such a metal–base pair would contain an additional crosslinking ethylenediamine linker which would create unusually stable base pairs. In contrast to all other metal–base pairs, formation of the metal–salen base pair **19** in DNA requires the addition of two components to the double strand. The first component is ethylenediamine (en), which reacts with the oppositely arranged salicylic aldehydes (S) in an equilibrium reaction to give the crosslinking salen ligand inside the duplex. The second additive is a metal ion, which is

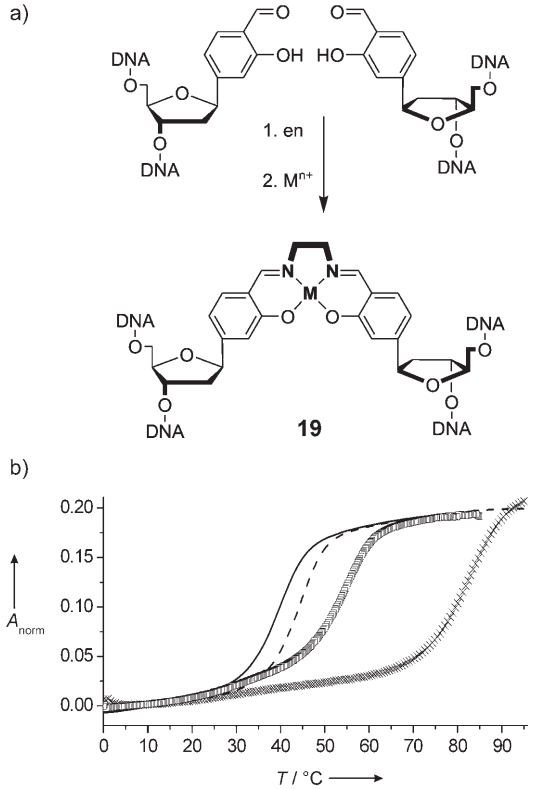


Figure 10. a) Formation of the metal–salen base pair **19** inside a DNA duplex. b) Comparison of the melting curves of the sequence d(5′-CACATTASTGTTGTA-3′)·d(3′-GTGTAATSACAACAT-5′): 1) without additives (39.9 °C, solid line); 2) with only ethylenediamine (45.5 °C, dashed line); 3) with only Cu^{2+} (54.9 °C, open boxes); and 4) with ethylenediamine and Cu^{2+} (82.4 °C, crosses) (3 μM DNA, 150 mM NaCl, 10 mM CHES buffer).[82]

supposed to bind inside the chelating ligand (examined were Cu^{2+}, Mn^{3+}, VO^{2+}, Fe^{3+}, and Ni^{2+}).[80]

It was shown that the assembly process operates cooperatively. The diamine is first needed to form the ligand, while the coordinated metal prevents subsequently the hydrolysis of the imines in water. This feature is a significant difference between all previously reported metal–base pairs and the salen concept (Figure 10a).

Under the chosen conditions (3 μM DNA, 150 mM NaCl, 10 mM buffer) the unmodified double strand containing an AT base pair instead of the ligands S (salicylic aldehyde) had a melting temperature of 50.1 °C. The salicylic aldehyde base pair (SS) in a corresponding duplex were found to decrease the melting temperature by 9.0 K to 41.1 °C, similar to a typical mismatch. The combinations of one ligand S opposite any of the natural bases dA, dT, dG, and dC led to an even higher destabilization, possibly because of steric clashes inside the duplex. Addition of excess ethylenediamine to a solution containing the SS base pair DNA duplex increased the melting temperature by 4.8 K possibly because of the crosslinking effect. The effect is rather small because the imine formation is highly reversible in aqueous solutions causing rapid hydrolysis of the crosslink during the melting process.[81]

More important, however, was the observation that the subsequent addition of one equivalent of Cu^{2+} resulted in a tremendous duplex stabilization (Figure 10b). One equivalent of Cu^{2+} induced a shift of the melting temperature to 82.4 °C, which is a shift of more than 30 K with respect to a normal AT base pair (+42.5 K with respect to the duplex containing the SS base pair). Additional Cu^{2+} had no further effect. To the best of our knowledge, this is the most dramatic increase in duplex stabilization ever observed with a metal–base pair. Addition of one equivalent of Mn^{2+} (which is known to be oxidized to Mn^{3+} upon complexation by salen ligands) increased the melting temperature again dramatically by 28.1 K to a final value of 68.8 °C.[82]

The hydroxypyridone ligand **12** used by Tanaka et al. in the same sequence context d(5′-CACATTA*H*TGTTGTA-3′)·d(3′-GTGTAAT*H*ACAACAT-5′) induced a stabilization of only 13 K when Cu^{2+} was added.[69] In contrast, the assembly of the copper–salen base pair **19** increased T_M by more than 40 K.[82,83] The value measured by Tanaka et al. is very similar to the value measured with the SS base pair duplex in the presence of Cu^{2+} but in the absence of ethylenediamine. This shows impressively how the ethylenediamine and the metal coordination act in concert to stabilize the duplex structure. In fact, many of the DNA duplexes containing the metal–salen complex can be purified by chromatography without any denaturing into single strands.[80]

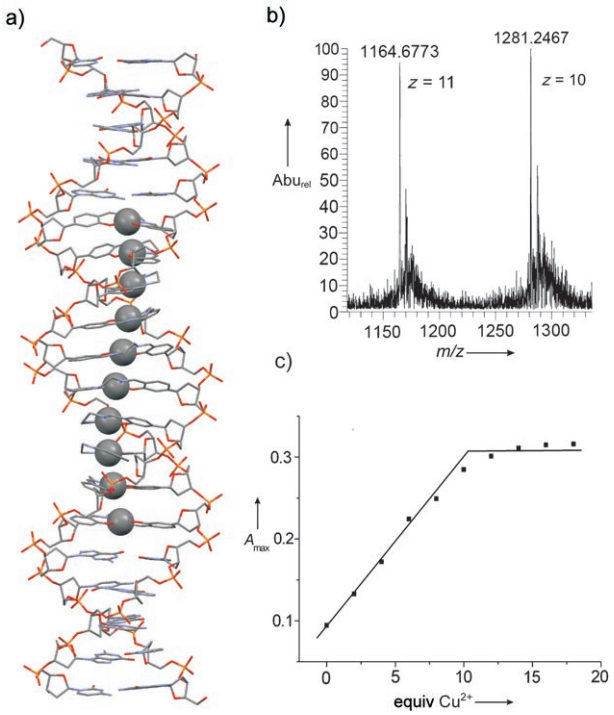


Figure 11. a) Computer model representing a plausible structure of ten metal–salen complexes **19** assembled inside the DNA double helix.[85] b) ESI-ICR mass spectrum of the duplex containing ten Mn^{3+} ions. The measured *m*/*z* values are in excellent agreement with the calculated molecular weight for [(5′-CGGCCSSSSSSSSSSCGCGC-3′)·(3′-GCCGGSSSSSSSSSSGCGCG-5′) + 10en + 10Mn^{3+} −20H_2O −30H^+]. c) Plot of the absorption maximum A_{max} of the copper–salen system (λ = 360 nm) versus the ratio [Cu^{2+}]/[duplex].[84]

3.6. Metal Stacking with the Salen–Base Pair

Taking advantage of the superior duplex stability provided by the metal–salen base pair, a stack of ten metal ions inside the DNA duplex was subsequently achieved (Figure 11a). In this structure, all natural base pairs within a complete turn of the double helix, extending over a distance of approximately 3.4 nm, are substituted by artificial metal-chelating base pairs.[84] The correct assembly of ten metal–salen complexes inside a double strand containing ten consecutive pairs of salicylic aldehydes was proven by ESI-ICR mass spectrometry (Figure 11b as an example for ten stacked Mn^{3+} ions inside the duplex) and UV-based titration experiments (Figure 11c as an example for the stacking of ten Cu^{2+} ions).

It became apparent that the metal–salen concept works best when the complexes inside the duplex feature a certain degree of kinetic lability. The reversibility of the complex formation is required to allow the system to break up intermediates, which seem to be formed initially under kinetic control, in order to reach finally the thermodynamic equilibrium, which seems to be the duplex. In this way, first a dynamic library of metal–DNA complexes is formed which converges with time to form the metal stack inside the DNA duplex.

3.7. Programmable Mixing of Metal Ions inside DNA

The ability to stack up to ten metal ions inside DNA raised the question if it would be possible to mix different metals in a programmable fashion inside the duplex, which would give

basically a metal-ion-based artificial genetic code. To this end a set of at least two different orthogonal ligand systems with sufficient selectivity for different metal ions had to be incorporated. Two systems were simultaneously developed, which both utilized the same two metal ions (Cu^{2+} and Hg^{2+}).[86] The incorporated ligands and the assembly strategies are, however, different. The Shionoya lab incorporated the hydroxypyridone ligand **12** for the complexation of Cu^{2+} and the Py–Py base pair **11** for Hg^{2+} into DNA. We, in contrast, inserted the salen ligand **19** to complex Cu^{2+} and the thymine–thymine mismatch **1** to coordinate Hg^{2+}. In the Shionoya system, the single strands contain only one natural base on either end. These strands are hence unable to form a duplex structure in the absence of any metal ions. We flanked the metal-stack region with two GC sequences on both ends five base pairs long to drive the equilibrium process towards duplex formation over potential hairpin generation. Hybridization of the double strands prior to metal addition gives a stable duplex with empty coordination sites which establishes a preorganized scaffold for the complexation process (Figure 12).

Complexation of two different kinds of metal ions in the two systems was followed by CD-spectroscopic titrations, and the final products were analyzed by high-resolution ESI mass spectrometry. It could be shown in both systems that the appropriate number of Cu^{2+} and Hg^{2+} ions were indeed coordinated inside the DNA strands. In the largest system, realized with the salen concept, a total of ten metal ions ($5\,Cu^{2+}$ and $5\,Hg^{2+}$) were assembled to give a mixed-metal-ion stack inside the DNA helix. Although no explicit proof for the sequence of the two different metal ions in the stack was provided, the sequence of the different coordinating bases (salicylic aldehydes and TT mismatches) likely determines the sequence of the different metal ions, making it possible to program the sequence of the metal ions inside the duplex. With both systems two different kinds of metal ions can be complexed in a programmable fashion at the atomic level inside the duplex.[86]

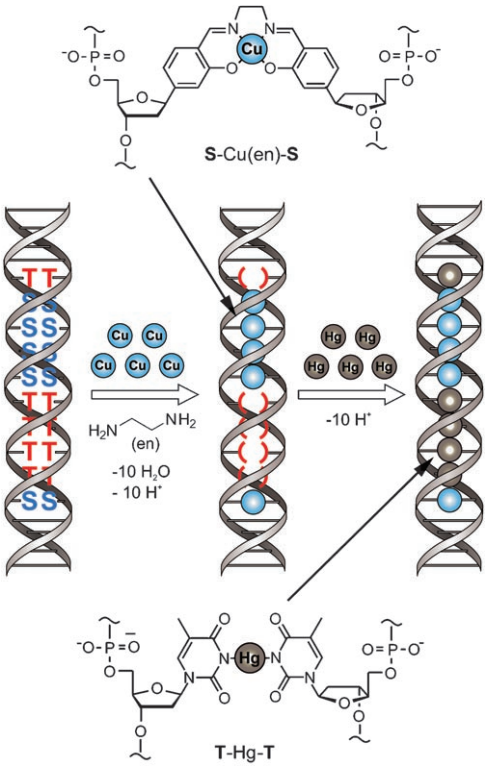


Figure 12. Generation of a mixed-metal stack with a determined sequence inside a DNA duplex by using pairs of salicylic aldehydes (SS) for the formation of Cu^{2+}–salen base pairs (**19**) in combination with thymine–thymine mismatches (**1**, TT) for the complexation of Hg^{2+}. The sequence is (5′-CGGCC*TSSSSTTTTS*CGCGC-3′) ·(3′-GCCGG*TSSSSTTTTS*GCGCG-5′).[86]

4. Conclusion and Outlook

A comparison of the metal–base pairs known so far is given in Table 1. Most reported duplex stabilizations caused by metal coordination are in the range of +5 to +20 K for

Table 1: Comparison of the discussed metal–base pairs.

Metal–base pair	Highest ΔT_M [K][a]	Methods[b]	Metal	M_{max}[c]	Ref.
1 (T-T)	+10 (AT: +3.0)	T_M, Tit_{UV}, Tit_{CD}, NMR, ESI-MS	Hg^{2+}	5	[46]
6 (Dipic-Py)	n.d.[d] (AT: −2.6)	T_M, EPR, CD, X-ray	Cu^{2+}	4[e]	[63, 64, 67]
7 (Dipam-Py)	+15.0 (AT: +3.9)	T_M	Cu^{2+}	4[e]	[64]
9 (SPy-SPy)	+19.1 (AT: +3.4)	T_M	Ag^{+}	3 (isol.)	[65]
10 (Spy-Py)	+11.5 (AT: −4.1)	T_M	Ag^{+}	3 (isol.)	[65]
11 (Py-Py)	+6.8 (AT: −5.5)	T_M, NMR	Ag^{+}	1	[68]
12 (H-H)	+13.1 (AT: +5.9)	T_M, Tit_{UV}, Tit_{CD}, EPR, ESI-MS	Cu^{2+}	5	[69, 73]
14 (dHQ-dHQ)	+28.9 (AT: +23.7)	T_M	Cu^{2+}	1	[71]
15 (pHQ-pHQ)	n.d.[d] (AT: +29.2)	T_M, CD	Cu^{2+}	1	[71]
16 (Bipy-Bipy)	+7.5 (AT: n.d.)	T_M, UV	Cu^{2+}	1	[75]
17 (PyA-PyA)	+18.1[f] (AT: +9.8)	T_M	Ni^{2+}, Co^{2+}	3[e]	[78]
18 (PyC-PyC)	+16.5[f] (AT: +4.4)	T_M	Ni^{2+}, Co^{2+}	1	[79]
19 (S-S)	+42.5[g] (AT: +32.3)	T_M, Tit_{UV}, Tit_{CD}, EPR, ESI-MS	Cu^{2+}, Mn^{3+}, VO^{2+}, Fe^{3+}, Ni^{2+}	10	[80, 82, 84]

[a] Highest reported values for strands containing one metal–base pair. First value: stabilization upon addition of a metal ion. In parenthesis: stabilization relative to a native AT base pair. Care must be taken when comparing the measured duplex stabilizations for different metal–base pairs because partly different sequences, buffers, and concentrations were used. [b] T_M = melting-temperature experiment (thermal de- and renaturing), Tit_{UV} = UV spectroscopic titration, Tit_{CD} = CD spectroscopic titration. [c] Maximum number of metal atoms per duplex. [d] No sigmoid melting curve without metal observed. [e] Presumed stacking of metals supported only by a single T_M value; no other characterization reported. [f] For Ni^{2+}. [g] For Cu^{2+}.

one incorporated metal–base pair. The highest duplex stabilization was obtained with the crosslinking copper–salen base pair. Geometrical comparisons of the artificial metal–base pairs with natural base pairs are in most cases not known. So far only the Schultz lab was able to obtain a crystal structure of the Dipic-Cu^{2+}-Py metal–base pair.[67] In the case of the metal–salen base pair, only a crystal structure of the Cu^{2+}-salen-nucleoside complex itself without DNA could be obtained which showed, however, an excellent geometrical match with a native Watson–Crick base pair.[80]

Almost 45 years ago, the first structural hypothesis for a metal-mediated base pair was developed by Katz. Today there is strong evidence that the structure that he proposed for the T-Hg-T base pair is indeed correct. In recent years, a number of structurally different ligand–nucleobase conjugates have been prepared and incorporated into oligonucleotides. The major common property of metal–base pairing is an enhancement of the thermal duplex stability. This feature might make metal–base pairing valuable for the constructuion of stable nanoarchitectures based on DNA. A combination of the metal–base pair concept with the well-established sequence-based techniques for the construction of complex DNA nanoarchitectures might eventually allow a convenient assembly of programmable constructs with several metal-binding sites spatially arranged in all three dimensions. This may open up interesting perspectives for molecular electronics and magnetism as well as for the synthesis and investigation of model compounds of multimetal enzymes. In this respect, the metal complexes inside the DNA might be used as enantioselective catalysts amenable to optimization by evolutionary methods.[87] All oligonucleotides discussed in this review have been prepared by automated solid-phase synthesis. An enzymatic approach starting from a triphosphate of the ligand nucleobase might allow the synthesis of much longer metal-complex-containing duplexes. Hirao et al. recently have shown that an artificial hydrophobic base pair can indeed be efficiently replicated by PCR and even transcribed from a DNA template into RNA.[88]

Another function that has been studied intensively in recent years is charge transfer through DNA. The two principal mechanisms of charge transport through DNA are: 1) transfer of positive charges ("holes")[54] and 2) transfer of excess electrons. Both processes have biological importance. Hole transfer is directly involved in the mechanism of DNA damage.[54,89] Electron injection into DNA was found to be a naturally occurring process for the repair of photodamages such as the TT dimer by the corresponding repair enzymes ("photolyases").[17,90] It turned out that the charge-transfer properties of unmodified DNA strands are too low to allow native DNA to be used as a true molecular electronic wires. Porath et al. connected DNA strands by nanoelectrodes and observed large-bandgap semiconducting behavior.[91] It is thus envisioned that a complete substitution of the interior of the double helix with metal ions might turn DNA into a good conductor.[92,86]

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salen base pair was also checked under these conditions for better comparability, and it was found that lowering of the DNA and salt concentration led to a decrease of all measured absolute melting temperatures, but the difference between the T_M prior and after assembly of the metal–base pair became even larger.
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