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Metal–base pairing in DNA

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ABSTRACT

The use of DNA as a molecular wire in nanoscale electronic architectures would greatly benefit from its capability of sequence-specific self-assembly. Although single electrons and positive charges have been shown to be transmitted by natural DNA over a distance of several base pairs, the high ohmic resistance of unmodified oligonucleotides imposes a serious obstacle. Exchanging some or all of the Watson–Crick base pairs in DNA by metal complexes may solve this problem and evolve DNA-like materials with superior conductivity for future nano-electronic applications. The so-called metal–base pairs are formed from suitable transition metal ions and ligand-like nucleosides which are introduced into both of the two pairing strands by automated DNA synthesis. This review illustrates the basic concepts of metal–base pairing and highlights recent developments in the field.

1. Introduction

1.1. DNA nanotechnology

DNA is the carrier of genetic information in each organism. As the product of million years of evolution, it features a maximal density of functionality embedded in its framework. The outer surface of the double helix is lined by the negatively charged sugar–phosphate backbone providing superior water solubility. The nucleobases can be accessed and recognized through the major and minor grooves by DNA binding enzymes such as transcription factors in a sequence-dependent manner. The interior of the duplex is occupied by the parallel stack of aromatic nucleobases, paired with each other through hydrogen bonding. The base pair distance of 3.4 Å and the helical pitch of 36° per base pair results in a complete helix turn approximately every 10 base pairs for the predominant B-type conformation of DNA.

Furthermore, DNA is a remarkably stable molecule that allows handling in a relatively wide pH range and does not decompose when heated to 100 °C for a limited time.

Nowadays, short DNA strands are routinely synthesized by an automated solid-phase process with full control over the desired sequence. Due to the purely organic synthetic character of this method, the introduction of modified building blocks such as artificial nucleosides, unnatural backbones or end-groups for further attachment is easily achieved. However, the choice of a suitable protecting group strategy complying with the solid-phase synthesis and the subsequent transfer of the products to the aqueous media is required.

Additionally, the step from using short, completely artificial oligonucleotides to longer DNA strands of hundreds or thousands of base pairs is achievable through the use of the molecular biologists' toolbox. Thus, short modified DNA strands can be stitched together and ligated to form longer constructs using appropriate enzymes such as endonucleases and ligases. A fascinating development of recent years is the use of artificial nucleoside triphosphates in the automated polymerase chain reaction (PCR) applying specifically designed or evolved polymerases [1].

In contemporary research in bottom-up nanotechnology, DNA has become an important building block owing to its superior properties [2]. Among the various research activities in bottom-up nanotechnology, molecular electronics is currently a most vibrant field due to several reasons: (1) the ongoing miniaturization of electronic circuits (as described by "Moore's Law" [3]) is facing a limit in structure size using the classic silicon-based photolithographic processes, (2) new electro-functional organic building blocks such as light-harvesting and charge separating assemblies of future photovoltaic devices might require to be wired up with cables of molecular dimensions and flexibility, (3) since already a trend is visible that microchips emerge from being pure data processors to more complex devices such as sensors for small molecules or interfaces to biological systems, the integration of organic and especially bio-derived building blocks is desirable [4]. DNA-based molecular electronics may provide electrical conducting bio-compatible bridges to make contact between electronic circuits and nerve cells of living organisms.

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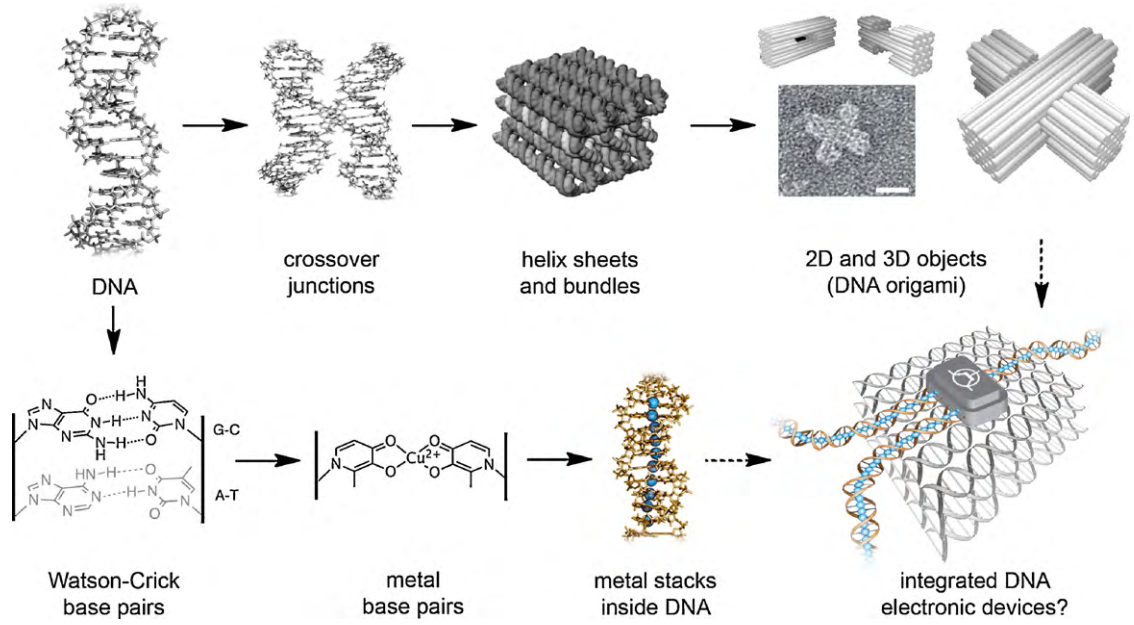

Fig. 1. Developments in the area of DNA nanotechnology. Upper row: generation of nano architectures from unmodified DNA strands following the rules of "DNA origami". Lower row: substitution of natural base pairs by metal base pairs has yielded stacks of metal ions inside the DNA double helix. Combined with DNA nano architecture, the self-assembly of integrated DNA electronic devices is imaginable (the upper right part of the image is reprinted by permission from Macmillan Publishers Ltd., Nature, 459, p414, copyright 2009).

The controllable sequence-dependent hybridization of DNA single strands to double strands and the possibility to generate junctions of several duplexes have been envisioned by some groups not many years ago to enable the creation of complex self-assembling 2- and 3-dimensional molecular architectures. In less than 30 years, these ideas have been realized and it turned out to be a rather simple task if certain rules in the design are followed. The field of DNA nanotechnology has been pioneered by the seminal works of Seeman [5], whereupon complex 2D structures were reported by Winfree and co-workers [6], Rothemund [7], Yan [8] and others [9]. By going into the 3rd dimension, the topic has recently been brought to another level of sophistication by researchers like Shih and co-workers [10], Gothelf and Kjems [11].

On the other hand, a plethora of synthetic DNA modifications have been realized in recent years such as artificial backbone structures, attachments of, for example, fluorescent dyes or surface anchors to the 3′ or 5′ ends or outside pointing functionalities that protrude from the sides of the double helix [12]. Most interesting, however, turned out to be the modifications that were introduced right into the core of the DNA double helix, thus replacing the natural base pairs. Among these artificial nucleobases, some were developed for studying the hydrogen bonding and stacking forces that hold together the double helix [13]. Others were incorporated into DNA to create a new set of base pairs with pairing abilities orthogonal to the natural Watson–Crick base pairs [14]. Again other artificial bases were developed for the analytical recognition of single nucleotide polymorphisms (SNPs) [15].

If natural DNA would be a superior conductor for electrons and functional building blocks such as molecular transistors could be incorporated into DNA strands by chemical synthesis, it would be possible to construct the first generation of self-assembling electronic circuits today (Fig. 1). However, it turned out that the electron conducting abilities of natural DNA seem to be not sufficient for the use of unmodified double strands in molecular electronic circuits. The reported conductivity values in the literature spread over a great range from 1 to 1×10^7 MΩ, a fact that was attributed to the differences in experimental techniques (contacting to the DNA, humidity, surface effects) and sequences studied [16].

Although the conductance of single excess electrons and positive charges through short patches of DNA is known to occur in natural processes such as DNA repair [17], the lossless conductance of many charges over distances of several hundred base pairs without destruction of the oligonucleotide is probably not possible. Furthermore, the transport of a steady current comprising a large number of charges along a molecular wire requires not only a low ohmic resistance but also a high robustness of the material used [18].

As a new approach to modify the electronic properties of DNA it was envisioned that doping the interior of oligonucleotide duplexes with metal ions may yield hybrid materials with enhanced conductivity or other interesting electronic effects [19]. First results in this direction have been published recently: Lee and co-workers presented a model system for a field-effect transistor based on M-DNA [20]. Joseph and Schuster studied the effect of a **T**–Hg–**T** base pair (see below) on the long-distance radical cation hopping properties but found no significant effect of this metal–base pair on the charge transport [21]. This observation is supported by a theoretical study of Voityuk who, on the other hand, proposes that a stack of **T**–Hg–**T** base pairs might play an important role in excess electron transfer [22]. Although not dealing with double-stranded DNA, the recent report on electrical transport through cation-stabilized G-quadruplex DNA of Erbe and co-workers is also noteworthy in this context [23].

In an alternative approach, DNA strands bound to surfaces are currently used as templates for the spatially controlled deposition of metal ions and subsequent formation of gap-free nanoscale wires by thermal soldering or photographic development. This topic will not be covered here and is reported elsewhere [24]. For the versatile roles of metal ions in naturally occurring nucleic acids the reader may refer to the following reference [25].

First experiments to introduce metal ions into the core of double helixes made use of unmodified DNA strands at high pH and metal ions such as Zn(II), Co(II) and Ni(II) [26]. The structure and conductive properties of this so-called M-DNA were, however,

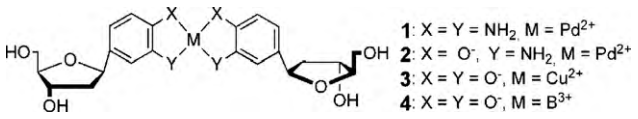

Fig. 2. The phenylenediamine, 2-aminophenol, and catechol metal–base pairs.

controversially discussed and despite some proposed structural models, the exact position of the metal ions inside or around the DNA strands remains unclear [19].

More reliable evidence for the binding of metal ions inside DNA duplexes consisting solely of natural base pairs was obtained for thymine-rich sequences upon addition of Hg(II) salts. As early as 1952 the effect of Hg(II) on DNA was studied and the formation and structure of a **T**–Hg–**T** metal–base pair, in which each thymine base is deprotonated at the N(3) position, was proposed in 1963 by Katz (**5** in Fig. 3, X = CH_3) [27]. Later, this observation was picked up again by Marzilli and Buncel who found evidence for the binding of Hg(II) to pairs of thymine bases by NMR experiments [28]. Recently, Ono picked up this principle again and contributed further studies on the **T**–Hg–**T** metal–base pair [29].

A clear indication for the interstrand binding of metal ions to oppositely arranged nucleosides is the observation of a stabilizing effect on the duplex structure in terms of an increase in the melting temperature T_M (see below). Whereas **TT** mismatches are known to strongly destabilize the DNA double helix, the addition of Hg(II) to sequences containing one or more **TT** mismatches leads to a significant rise of the duplexes thermal stability as seen in the DNA melting profiles. In a similar approach, the analogous **U**–Hg–**U** metal–base pairs (**5** in Fig. 3, X = H) were realized in RNA by Müller and co-workers [30]. Noteworthy is furthermore the observation of a Au(III) ion bound inside a **GC** base pair (with guanosine deprotonated at the N1 position) that was found in a crystallographic study aimed at screening the interaction of an RNA duplex with various metal ions [31].

Recently, Ono et al. reported the **C**–Ag–**C** metal–base pair **6** which forms upon addition of Ag(I) to double strands containing **CC** mismatches [32]. Müller et al. subsequently found evidence for the formation of parallel-stranded helices containing eight consecutive **C**–Ag–**C** base pairs by CD and UV measurements [33].

The fact that **TT** mismatches bind Hg(II) but no Ag(I) and **CC** mismatches show an opposite behavior was exploited by Willner and co-workers to create logic AND as well as OR gates based on metal binding oligonucleotides which are attached to quantum dots [34].

Since the binding capabilities of the natural nucleobases to metal ions were found to be limited to only a small number of metals and – depending on the sequence – Watson–Crick base pairing might interfere with metal binding, a new approach of metal–base pairing based on designed, artificial nucleobases with metal binding potential was developed.

In these "metal–base pairs", the nucleobase attached to the sugar is replaced by a ligand capable of coordinating a transition metal ion, mostly in a linear or square–planar fashion [19]. Inside

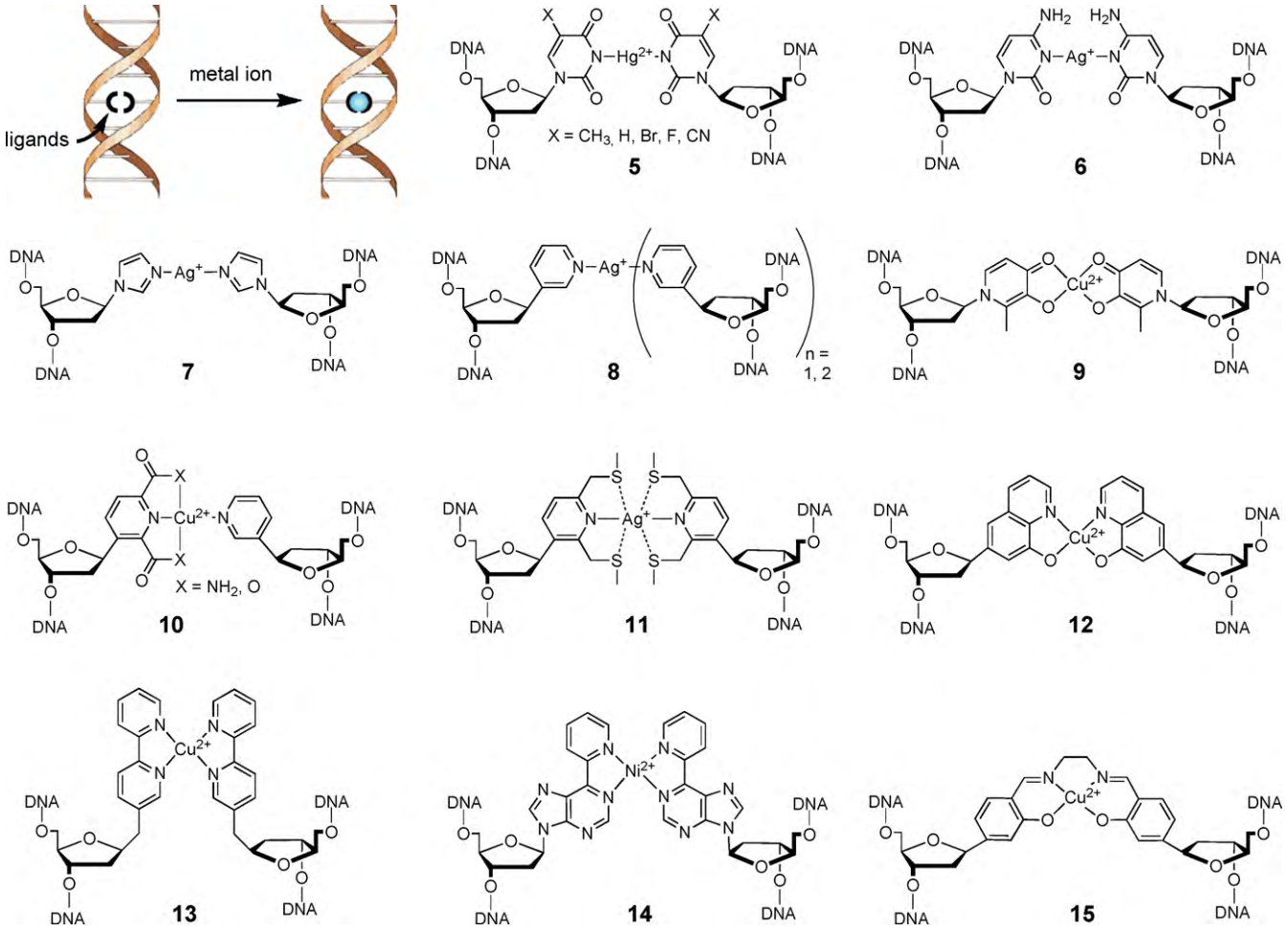

Fig. 3. Overview of most of the reported metal–base pairs today. Some derivatives are omitted and only one kind of metal ion is shown. Since no or only limited structural data is available for most of the depicted metal–base pairs inside DNA double strands, the real coordination geometries might differ from the structures drawn here. References not mentioned in the text: **5** (X = Br, F, CN) [41], **11** [42], **12** (also reported with a simplified propanediol backbone) [43], **13** [44], **14** [45].

the DNA double helix, the metal binding of two oppositely arranged ligands ideally results in a flat coordination complex that integrates as part of the base pair stack without distorting the shape of the duplex.

1.2. Overview of metal–base pairs

The field of metal–base pairing based on artificial nucleosides was pioneered by Tanaka and Shionoya who studied the coordination chemistry of monomeric ligand-modified nucleosides (**1**–**4**, Fig. 2) in aqueous solutions and so established the foundation of the incorporation of ligands inside the DNA double helix [35].

However, it proved difficult to incorporate the oxidation sensitive catechol and ortho-phenylene diamine compounds **1**–**4** into oligonucleotides and metal–base pairing inside DNA double strands could not yet be realized in these early experiments. Shortly following this first work, the groups of Schultz and Shionoya both succeeded in using the known pyridine nucleoside **P** for metal-mediated base pairing inside the DNA double helix. The Shionoya group found a way to coordinate Ag(I) ions between two pyridine nucleosides inside a DNA duplex and could show the formation of triple-stranded systems based on this approach (**8** in Fig. 3, see also Section 2.1) [36]. Meggers and Schultz developed an unsymmetrical metal–base pair consisting of one pyridine base **P** and one pyridine ligand equipped with two extra coordinating groups resulting in a family of metal–base pairs denoted as **10** in Fig. 3 [37]. Two of the **Dipic**–Cu–**P** base pairs (**10** with X = O) were successfully incorporated into the dodecamer sequence [d(5′-CGCG**Dipic**AT**P**CGCG)$_2$] and the structure was elucidated by X-ray crystallography showing Jahn–Teller distorted octahedral coordination of the Cu(II) ions between the **P** and **Dipic** ligands forming the metal–base pair and two oxygen atoms of the neighboring nucleotides [38].

In the following years, a variety of further metal–base pairs were developed (Fig. 3). Since only selected examples will be discussed here, the reader may refer to preceding review articles [19,39,40] and the respective original publications referenced in the caption to Fig. 3.

The procedure for the synthesis and incorporation of ligand-modified nucleosides is roughly sketched in Fig. 4 [46]. The stepwise solid-phase synthesis of oligonucleotides following the established protocols requires each nucleotide to be incorporated as a 3′-phosphoramidite with a 5′-dimethoxytrityl protecting group. While the natural nucleosides are commercially available, the ligand-modified nucleosides have to be synthesized. The key step in the synthesis of artificial nucleosides is the coupling of the nucleobase (or ligand) with the deoxyribose sugar moiety. The examples in Fig. 3 show that either a C–N bond or a C–C bond has to be formed and several methods for either case have been successfully implemented [47]. The glycosidation is followed by the separation of the usually desired β-anomer from the α-anomer and subsequent standard protecting group operations to obtain the building blocks for the automated DNA synthesizer. Furthermore, it may be necessary to carefully choose a suitable protecting group strategy for the ligand which is compatible with DNA synthesis but allows for a smooth deprotection of the artificial nucleobases after DNA synthesis in aqueous solution.

After the cyclic DNA synthesis process, the oligonucleotides are cleaved from the solid support, fully deprotected and dissolved in an aqueous buffer. The single strands may be purified by HPLC or other techniques, characterized by mass spectrometry and their concentration is estimated by UV spectroscopy (requiring to know the molar extinction coefficient of all included nucleotides). Double strands are obtained by mixing equimolar amounts of matching single strands in an aqueous solution containing an electrolyte such as NaCl of high ionic strength and a buffer substance to adjust the required pH. The metal–base pairs are finally assembled by adding the needed amount of metal ions which can be monitored by UV- or CD-based titrations, mass spectrometry and thermal denaturation experiments. In the latter experiment, the extinction at the absorption maximum of the nucleobases at 260 nm (which is higher for single strands than for the double strand) is plotted against the temperature of a solution of the DNA (for example, see Fig. 6). The transition point of the curve is defined as the melting point T_M of the double-stranded DNA and is dependent on the length, sequence and concentration of the DNA as well as the nature and concentration of the additives (salt, buffer).

The stabilizing effect of metal ions on the DNA strands containing oppositely arranged ligands can be expressed as the difference ΔT_M between the melting points of the duplex before and after addition of the respective metal ions. Care has to be taken when duplex stabilizing effects in terms of ΔT_M are compared for the different metal–base pairing systems described in the literature

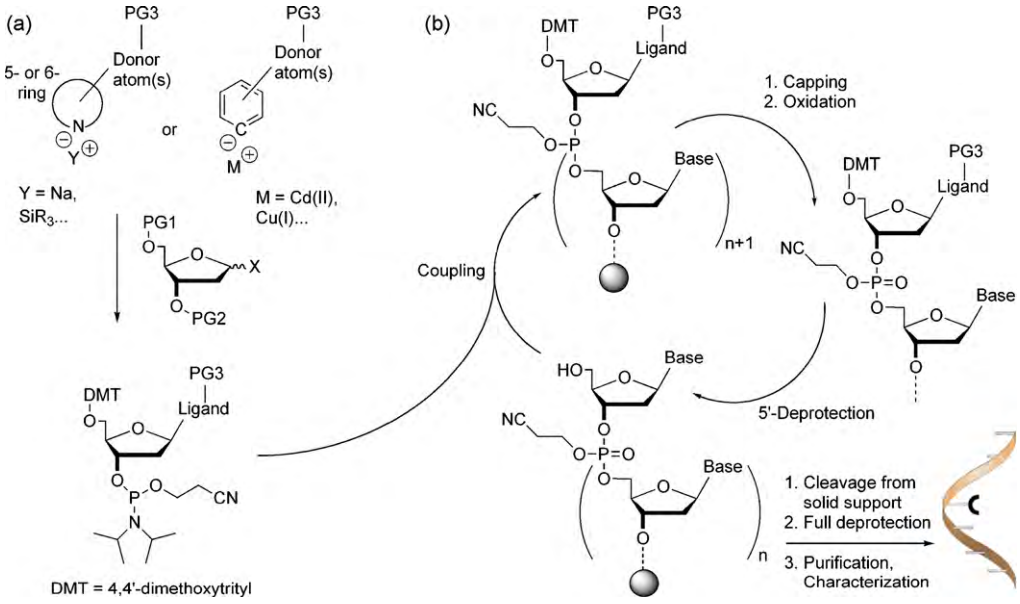

Fig. 4. Synthetic strategy to ligand-modified nucleosides and incorporation of artificial nucleosides into oligonucleotides by automated solid-phase synthesis using phosphoramidite building blocks.

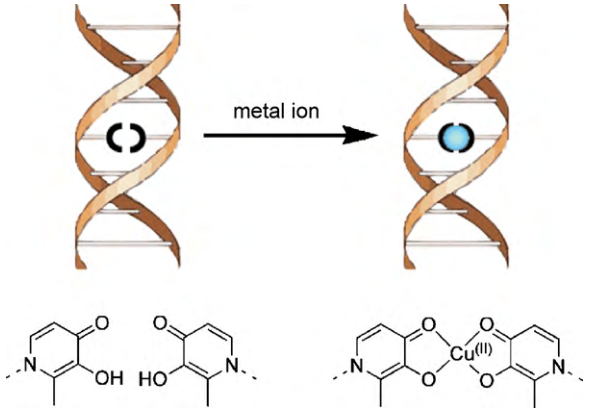

Fig. 5. The hydroxypyridone base pair.

because the melting temperature T_M is not a thermodynamic value. However, its easy determination and descriptive character have made it a common parameter to describe thermal duplex stability.

2. Selected examples

2.1. The hydroxypyridone metal–base pair

3-Hydroxy-4-pyridone is a bidentate chelate ligand capable of forming square–planar 2:1 complexes with metal ions such as Cu(II) and octahedral 3:1 complexes with metal ions such as Fe(III). Attached via its nitrogen atom to the C1′ position of deoxyribose, a hydroxypyridone nucleoside **H** (**9** in Fig. 3; carrying a methyl group at the 2-position for purely synthetic reasons) was synthesized and incorporated into oligonucleotides by Tanaka and Shionoya et al. [48]. Addition of Cu(II) to a duplex containing two oppositely arranged hydroxypyridone bases was shown to result in the formation of an **H**–Cu–**H** metal–base pair inside the double helix (Fig. 5).

In the absence of transition metal ions, the hydroxypyridones lead to a destabilization in respect to an **AT** or **GC** base pair at the same position. Upon formation of one metal–base pair inside the DNA double strand, the duplex stabilization ΔT_M was estimated to be 13 K in the given sequence context (Fig. 6) [48]. Addition of Cu(II) to a similar sequence containing an **AT** base pair instead of the hydroxypyridones did not result in any change of T_M.

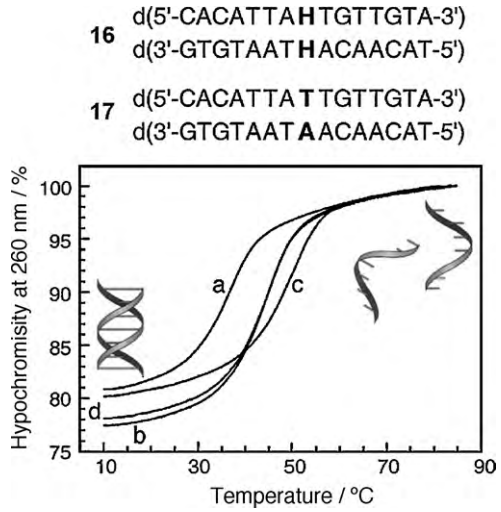

Fig. 6. Melting curves of the duplexes **16** (a and c) and **17** (b and d). [**16**] = [**17**] = 2.0 μM in 10 mM sodium phosphate buffer, 50 mM NaCl (pH 7.0). (a) and (b) no Cu^{2+}; (c) and (d) $[Cu^{2+}]$ = 2.0 μM.

The system was further characterized by ESI mass spectrometry, UV-based titration studies and CD spectroscopy, all indicating the complexation of one Cu(II) ion inside the double strand. An X-ray structure of the monomeric **H**–Cu–**H** metal–base pair **9** confirmed the square-planar coordination of the central Cu(II) ion by two hydroxypyridone ligands [48]. Without being constrained by the surrounding DNA structure, the ligands, however, were found to be coordinated in an *anti*-configuration as opposed to the *syn*-configuration that was anticipated for the **H**–Cu–**H** metal–base pair inside DNA by molecular modeling. Recently, this expected *syn*-configuration was confirmed by a crystal structure containing an **H**–Cu–**H** metal–base pair **9** inside a duplex, albeit with a modified backbone [49].

In a related study aiming at the coordination of soft metal ions such as Pd(II) by the Shionoya and co-workers, two sulfur containing ligands based on hydroxypyridone **H** in which the two coordinating oxygen atoms are exchanged one by one for a sulfur atom were synthesized and tested for their metal binding abilities [50]. Although the efficient formation of Pd(II) and Pt(II) metal base pairs could be achieved using the monomeric nucleosides, the incorporation into DNA duplexes has not been reported yet.

The incorporation of up to five **H**–Cu–**H** metal–base pairs **9** in a way that metal ion stacks are formed inside the double strand is discussed in Section 3.

Another interesting feature of the hydroxypyridone ligand that was utilized recently in the DNA context is its aforementioned capability of forming 3:1 octahedral complexes with metal ions such as Fe(III) (Fig. 7) [51]. Mixing 3 equiv. of a tetrameric $\mathbf{H}_4$ sequence with 4 equiv. of Fe(III) ions resulted in the formation of a presumably triple helical complex $(\mathbf{H}_4)_3Fe_4$ after 2 days at 85 °C as shown by the UV-based titration studies, CD measurements and ESI mass spectrometric data. This phenomenon may be exploited in future for the interconversion of duplex to triplex structures based on the same ligand and for the construction of branched metal DNAs.

Noteworthy in the context of triple helix formation is also the earlier work of the same group using the pyridine ligand inside the duplex [d(3′-T_{10}**P**T_{10}-5′)·d(3′-A_{10}**P**A_{10}-5′)] to enable the binding of another single strand d(3′-T_{10}**P**T_{10}-5′) in the fashion of a heterotriplex upon addition of Ag(I) ions (**P** = pyridine in base pair **8**, Fig. 7) [36] and the recent observation of parallel triplex stabilization by Ag(I) ions reported by Jyo and co-workers [52].

2.2. The salen–metal–base pair

Whereas the assembly of most metal–base pairs can be understood as a two-component process comprising the DNA duplex containing the preorganized ligands as one reaction partner and the metal ion as the second component, the case is different for the salen–metal–base pair **15** (**S**) introduced by Clever, Carell and co-workers [53]. The assembly of the salen–metal–base pair inside the DNA double helix requires the addition of two components to the duplex containing a pair of salicylic aldehydes at the facing positions. The first additive is ethylenediamine which reacts with both aldehyde groups under elimination of two molecules of water to the well-known salen ligand (Fig. 8). Since the formation of imines is reversible in water, an excess of ethylenediamine is added in order to drive the equilibrium to the side of the salen ligand. Subsequently a transition metal ion such as Cu(II), Mn(II) (oxidized to Mn(III) upon complexation), Fe(III) or vanadyl (VO^{2+}) is added and the metal–base pair **15** is formed [53b].

The local structure of the metal–base pair was elucidated by X-ray analysis of a monomeric Cu(II) complex of the salen ligand that was formed from the free salicylic acid nucleoside [53b]. The structure confirms the square-planar coordination of the metal ion. A superposition of the molecular structure of the salen–Cu(II) metal–base pair with a natural, hydrogen-bonded Watson–Crick base pair

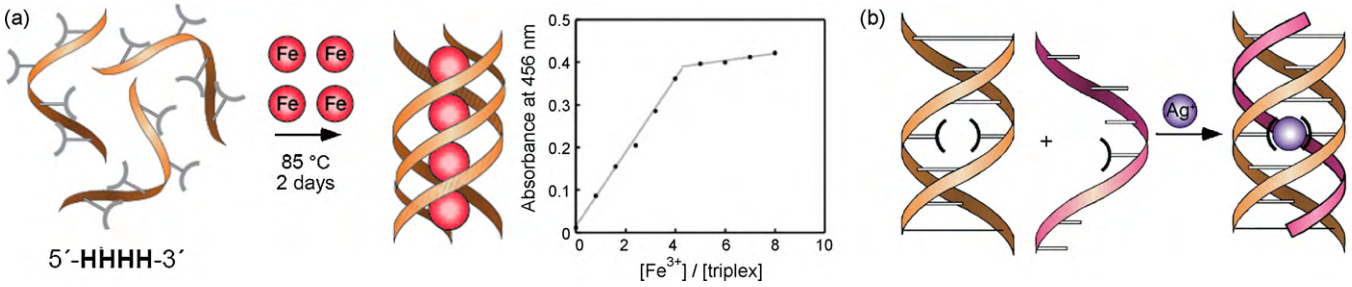

Fig. 7. Formation of triple helices. (a) From 3 equiv. of $\mathbf{H_4}$ strands and four Fe(III) ions. (b) A metal-containing triple helix can also be formed upon addition of Ag(I) ions to a mixture of duplex [d(3′-T_{10}**P**T_{10}-5′)·d(3′-A_{10}**P**A_{10}-5′)] and single strand d(3′-T_{10}**P**T_{10}-5′).

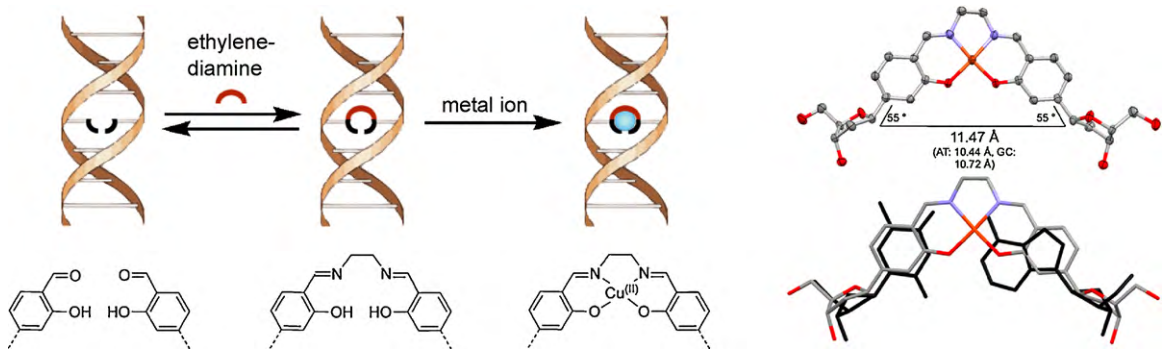

Fig. 8. (a) Reversible formation of the salen ligand inside the DNA and subsequent coordination of the metal ion giving the salen-metal–base pair. (b) X-ray structure of the monomeric salen–Cu(II) metal–base pair and superposition with a Watson–Crick AT base pair.

is shown in Fig. 8b. The good structural agreement between the natural **AT** base pair and the salen–metal–base pair suggests a tremendously good fit of the salen–metal–base pair inside the DNA double helix [53b]. This assumption could be supported also by CD measurements of salen–metal-containing DNA strands [53a].

The determination of the thermal duplex stabilization by thermal denaturation experiments (using the same sequence as Shionoya et al. [d(5′-CACATTA**S**TGTTGTA-3′)·d(3′-GTGTAAT**S**ACAACAT-5′)]) led to a surprising result. Whereas the duplex containing the two salicylic aldehydes **S** has a melting temperature T_M of 40 °C (about 10 K destabilization compared to AT), the addition of ethylenediamine and Cu(II) resulted in formation of the salen–Cu(II) metal–base pair (**15** in Fig. 3) accompanied by a tremendous and unprecedented increase in T_M to 82 °C (ΔT_M = +42 K, Fig. 9) [53a].

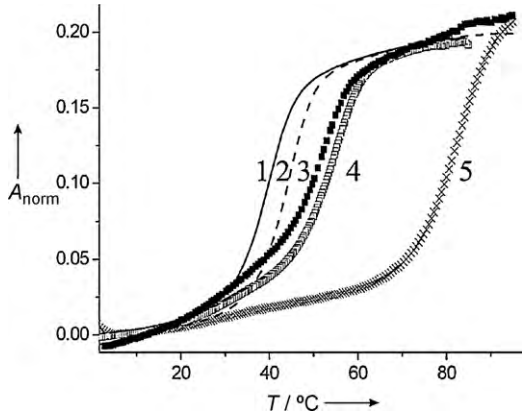

Fig. 9. Comparison of the melting curves of the sequence d(5′-CACATTA**S**TGTTGTA-3′)·d(3′-GTGTAAT**S**ACAACAT-5′) (1) without any additives (39.9 °C, solid line); (2) with only ethylenediamine (45.5 °C, dashed line); (3) with methylamine and Cu(II) (52.3 °C, filled boxes) (4) with only Cu(II) (54.9 °C, open boxes) and (5) with ethylenediamine and Cu(II) (82.4 °C, crosses) (3 μM DNA, 150 mM NaCl, 10 mM CHES buffer).

The reason for this large duplex stabilization was elucidated by a stepwise melting point analysis of the single components added to the DNA duplex. If only ethylenediamine was added, the melting point T_M was raised by only 5 K, attributable to the reversible formation of the imine cross-link in water. If only Cu(II) was added to the duplex, T_M was raised by 15 K, a value comparable to the examples of duplex stabilization reported for the other metal–base pairs. However, the high stabilization of ΔT_M = +42 K was achieved only when both, the ethylenediamine and the Cu(II) ions were added to the duplex [53a]. A reasonable explanation is that the formation of the salen system from the salicylic aldehydes and ethylenediamine provides an excellent coordination environment for the metal and the binding of the Cu(II) ion to the tetradentate chelate ligand results in a stabilization of the imine bonds towards hydrolytic cleavage. This cooperative binding effect is responsible for the tremendous duplex stabilization because a cross-link is formed that is of far more strength than metal coordination alone (Fig. 10). Supporting this hypothesis is further the observation, that the addition of Cu(II) and methylamine instead of ethylenediamine resulted in a stabilization ΔT_M of only about 12 K which is comparable to the value obtained for the DNA sample containing Cu(II) alone [53a].

In the case of the Cu(II)-containing duplex, the thermal de- and renaturing profiles (heating, cooling curves) are superimposable. The measurements of the same sequence containing ethylenediamine and Mn(III), however, reproducibly showed a strong hysteresis between the de- and renaturing profiles (not shown).

This can be explained with the thermal instability of the salen–Mn(III) complex when exposed to temperatures above T_M for elongated times (Fig. 11). In these cases, the transition in the heating curve can be assigned to the metal-containing and thus higher melting duplex, whereas the cooling curve shows that the duplex re-hybridizes before reincorporation of the metal (expressed by a lower T_M).

However, after the sample is allowed to spend some time at a temperature below T_M, the metal is again fully incorpo-

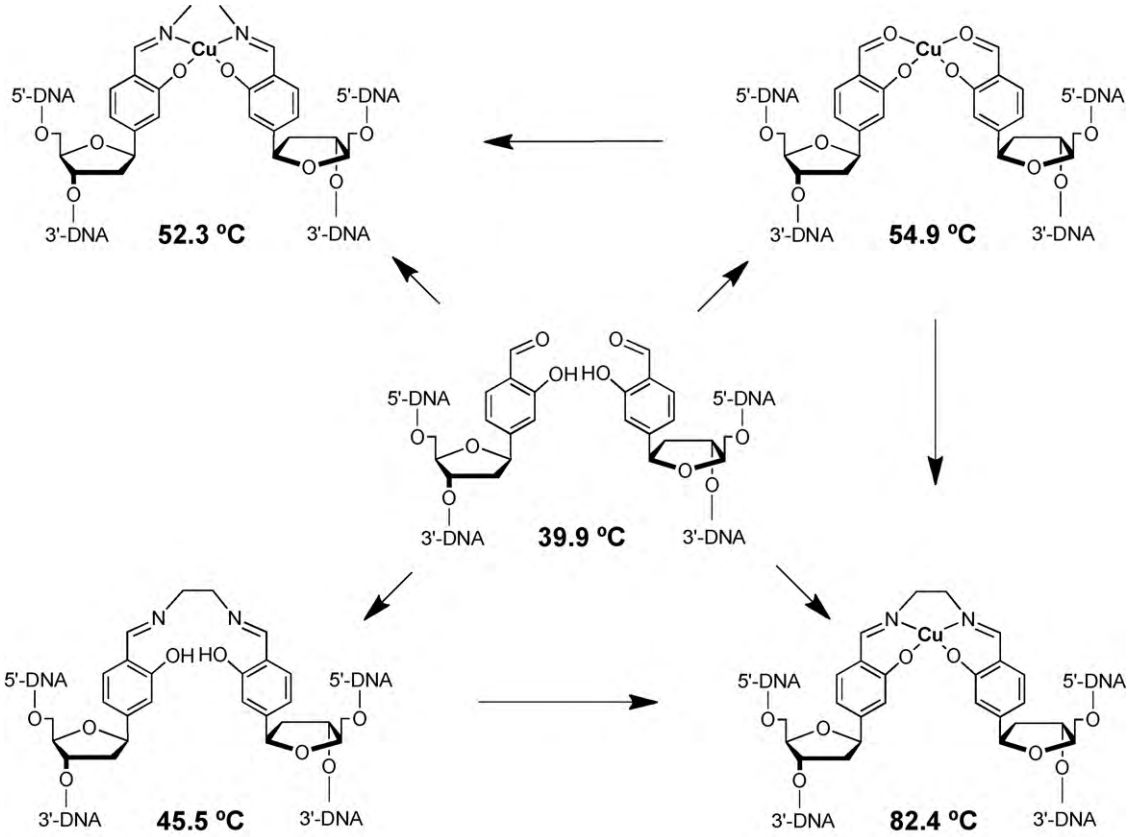

Fig. 10. Cooperative assembly of the salen metal–base pair in DNA.

rated giving rise to a denaturing profile superimposable with the preceding denaturing curve. The conclusion can be drawn that the salen–Cu(II) metal–base pair **15** is more stable than the salen–Mn(III) metal–base pair even at high temperatures. This fact is corroborated also by the higher ΔT_M caused by the addition of Cu(II) (+42 K) in comparison to the addition of Mn(III) (+28 K).

Another form of cooperative binding of Cu(II) ions is observed in the case of duplex d(5′-CACATT**SS**TGTT GTA-3′)·d(3′-GTGTAA**SS**ACAACAT-5′) comprising two neighboring salen ligands [53c]. The addition of 0.5 equiv. of Cu(II) results in a 1:1 mixture of metal-free duplexes and duplexes containing two Cu(II) ions as observed by a thermal denaturation study (Fig. 12). The binding of the first Cu(II) ion to the duplex enhances the affinity for binding of the second Cu(II) ion so that all available Cu(II) ions in the solution end up being incorporated pairwise. At any given ratio of Cu(II) ions to DNA duplexes (<2) the reaction mixture thus contains only two species: metal-free duplexes and duplexes containing two metal ions. Furthermore, this model is in accordance with the observation of isosbestic points in the titration curves of all examined double strands containing two or more adjacent metal binding sites such as **HH** or **SS** (see Section 3).

2.3. The triazole metal–base pair

The use of 5-membered heterocycles as artificial nucleosides with metal coordinating abilities such as imidazole, triazole and tetrazole was systematically studied by Müller et al. [40,54]. Prior to the incorporation of ligands such as **7** into DNA the ability to coordinate metal ions such as Ag(I) and Hg(II) in terms of the stability constant and the ligand to metal ratio of the formed complexes was examined [55]. Additionally, the pK_a values of the monomeric nucleosides were calculated and determined experimentally to estimate the proper pH range that is suitable for the specific formation of metal–base pairs in the oligonucleotide environment. Out of 5-membered heterocycles, the incorporation of 1,2,4-triazole **Z** into to DNA was chosen because of its ability to differentiate between Ag(I) and Hg(II) (Fig. 13).

A highly interesting observation was made, when the hybridization behavior of the palindromic sequence d(A_7**Z**$_3T_7$) containing three neighboring 1,2,4-triazole ligands **Z** was examined (Fig. 14) [54]. In the absence of metal ions, the strand forms a hairpin due to the destabilizing effect of the **Z** bases lacking any ability to engage in mutual hydrogen bonding. Upon addition of Ag(I) ions, however, the oligonucleotides undergo a structural transition and metal binding leads to dimerization which results in a double strand containing three stacked Ag(I) ions as evidenced by UV spectroscopic and MALDI-TOF mass spectrometric data. Whereas the hairpin exhibits a concentration-independent melting temperature owing to its unimolecular melting behavior, the melting curves of the sample containing Ag(I) ions showed a dependence on the oligonucleotide concentration which verifies the existence of a double strand. Furthermore, the proposed structural switching process was supported by fluorescence resonance energy transfer (FRET) experiments with dye-labeled strands of the same sequence and by the observation of an increase in molecular size in dynamic light scattering (DLS) experiments [54].

3. Stacking and mixing of metals inside DNA

After the prerequisites for the formation of metal–base pairs inside the DNA double helix using a variety of ligands and metal ions were elucidated, the focus of the research activities in the field of metal–base pairing has recently shifted to the realization of longer metal stacks inside DNA [56]. Whereas the examination

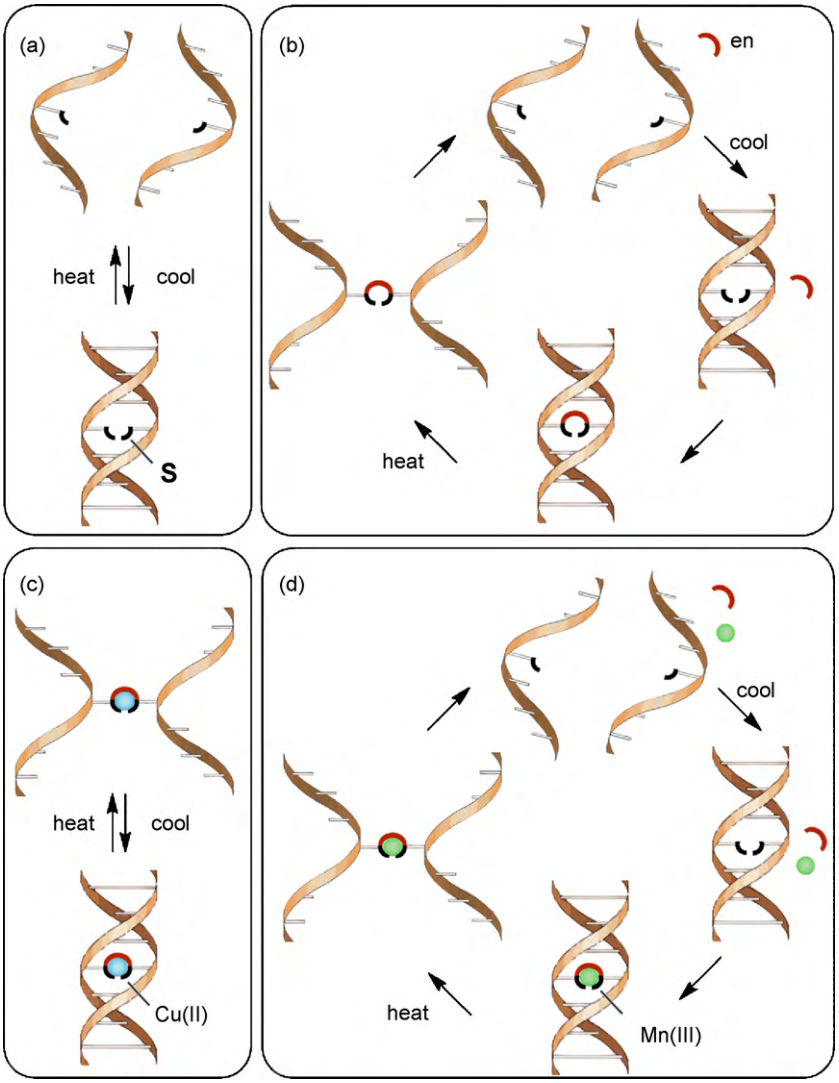

Fig. 11. De- and renaturation pathways of the **S**-containing duplexes with (a) no additives, (b) only ethylenediamine ("en"), (c) en and Cu(II) and (d) en and Mn(III).

of oligonucleotides containing only one metal–base pair is of great importance for determining basic parameters like binding constant and thermal duplex stabilization ΔT_M, the study of duplexes containing a number of directly stacked metal ions is supposed to contribute to the ultimate goal of the formation of molecular wires from modified DNA strands. In the course of examining the coordination of Hg(II) ions to **TT** mismatches, Marzilli reported the stacking of three Hg(II) ions inside the middle of a short oligonucleotide [d(5′-GCGC**TTT**GCGC-3′)]$_2$ (Fig. 15) [28b]. The extension to longer stretches of stacked metal ions, however, already failed in the case of the homologous sequence containing one additional thymine in row. The addition of Hg(II) ions to this strand resulted in the formation of a hairpin due to intrastrand cross-linking of the terminal **T** residues of the **T$_4$** stretch instead of duplex formation. In contrast to this, Müller and Sigel et al. recently presented conclusive evidence for the formation of RNA duplexes containing up to six consecutive **U**–Hg–**U** metal–base pairs (**5**, X = H) inside RNA duplexes by performing NOESY experiments with ^{15}N and ^{13}C labeled oligonucleotides [30].

A successful demonstration of the stacking of up to five Cu(II) ions inside a duplex formed from the artificial oligonucleotide [d(5′-G**H**$_n$C-3′)]$_2$ (n = 1–5) was achieved by Tanaka and Shionoya using the hydroxypyridone base pair **H** [57]. The exclusive formation of the double strand containing five Cu(II) ions was indicated by UV spectroscopic titration experiments, mass spectrometry and EPR experiments showing a ferromagnetic interaction between the stacked paramagnetic Cu(II) ions. The EPR experiment also allowed a rough determination of the distance between the metal–base pairs to be 3.7 ± 0.1 Å which would be comparable to the distance between the base pairs in natural DNA [57]. A subsequent theoretical DFT study by Pati et al. supported the ferromagnetic coupling in this **H**–Cu–**H** system (albeit assuming a Cu–Cu distance of 3.2 Å with the neighboring metals bridged by two oxygen atoms of the ligands in a {Cu_2O_2} convex quadrangle structure) [58]. The same theoretical work proposed an antiferromagnetic coupling for two neighboring salen–Cu(II) metal–base pairs **S**–Cu–**S** and indeed this anticipated interaction was found to occur by Schiemann et al. in a DNA double strand containing two adjacent salen–Cu(II) metal–base pairs [59]. Nakanishi et al. also contributed theoretical studies for both above-mentioned base pairing systems [60].

With 10 metal ions in a row, the double number of stacked metals was successfully incorporated inside double strands by using the salen ligand **S** introduced in Section 2.2 [53c]. The sequences were designed to consist of non-palindromic **GC** stretches at their ends in order to achieve preorganization of the 10 adjacent pairs of salicylic aldehydes and so facilitate the formation of the salen complexes [(5′-CGGCC**S$_{10}$**CGCGC-3′)·(3′-GCCGG**S$_{10}$**GCGCG-5′)]. The aforementioned differences in duplex stabilization of Cu(II)

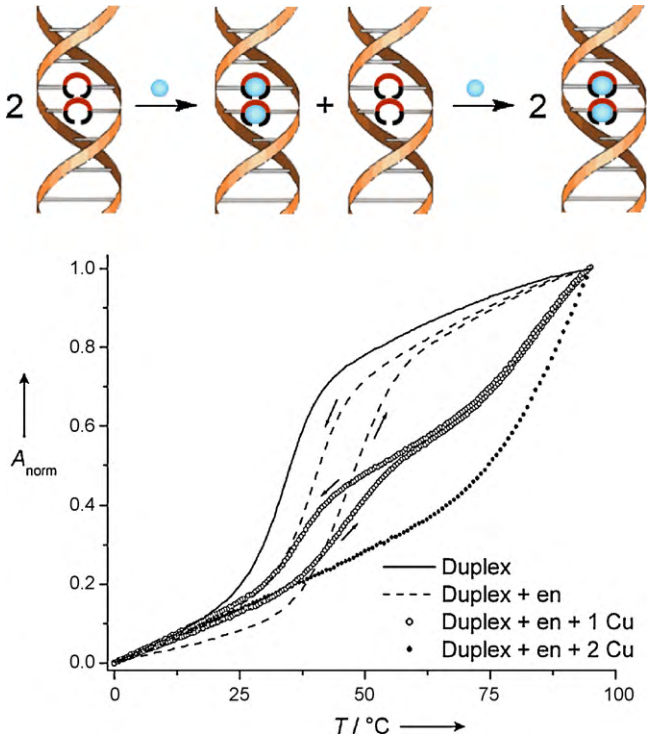

Fig. 12. The uptake of metal ions inside duplexes containing two neighboring salen ligands proceeds cooperatively: adding 0.5 equiv. of Cu(II) results in a 1:1 mixture of metal-free duplexes and duplexes containing two Cu(II) ions. Note the observed hysteresis effect which is characteristic for the melting of the duplex containing ethylenediamine but no Cu(II) ions [53c].

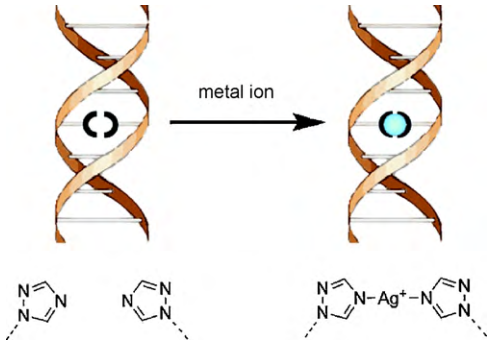

Fig. 13. Assembly of the Ag(I)-mediated base pair of Müller et al.

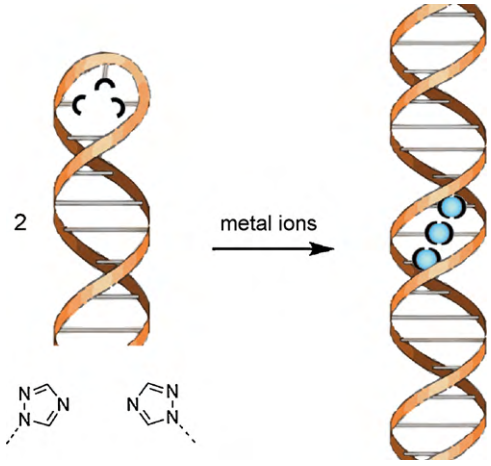

Fig. 14. Control of hairpin to duplex transition by metal addition.

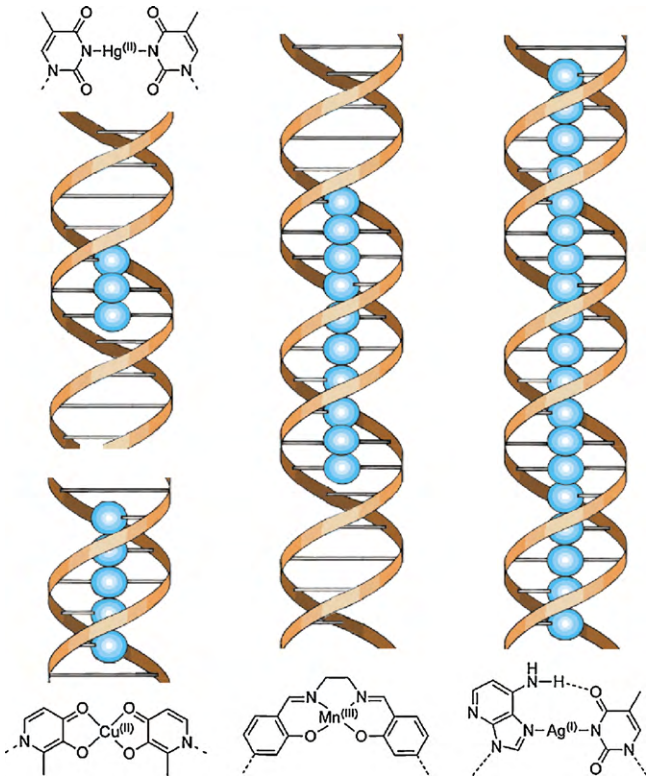

Fig. 15. Metal stacks inside the DNA double helix.

vs. Mn(III) were found to play an important role in the selective and clean formation of double strands containing a larger number of metal ions. Whereas the higher stability of the salen–Cu(II) metal–base pairs with respect to the salen–Mn(III) metal–base pair is desirable in systems containing only a single salen ligand, the choice of metal for the realization of longer stacks inside the DNA was found to follow an opposite trend. Due to the high stability of the salen–Cu(II) base pair, misfolded, unspecifically cross-linked or overlapping sequences were formed as kinetic products and lead to a relatively large distribution of signals in the ESI mass spectra around the expected value for the duplex containing 10 neatly stacked salen–Cu(II) complexes. In contrast, the addition of Mn(III) resulted in the formation of only a single product after the reaction mixture was allowed to equilibrate at room temperature for several days [53c]. This observation was ascribed to the "self-healing" capabilities of the salen–Mn(III) containing duplexes that cause initially formed kinetic products to transform into one thermodynamic product after some time.

The formed stacks of 10 metals inside the DNA double helix were characterized by UV-based titration experiments, high resolution ESI-FTICR mass spectrometry and CD spectroscopy.

By combining both hydrogen bonding and Ag(I) coordination in one base pair consisting of one thymine **T** and an artificial 1-deazaadenine **D**, Müller et al. succeeded in the stacking of 19 Ag(I) ions inside a 20-mer sequence [61]. The **D** and **A** bases thereby are thought to form a doubly hydrogen-bonded Hoogsteen base pair and the addition of Ag(I) ions leads to substitution of one of these hydrogen bonds as illustrated in Fig. 15. Also in this case, the rather low duplex stabilization observed for a single **D**–Ag–**T** metal–base pair was seen as an advantage for the error-free formation of long metal-containing double strands due to the required structural equilibration to reach the thermodynamically most favored structure. Recently, Müller and Sigel et al. reported the solution structure determination of a DNA double strand containing three consecutive imidazole–Ag(I) metal–base pairs **7** by a detailed NMR

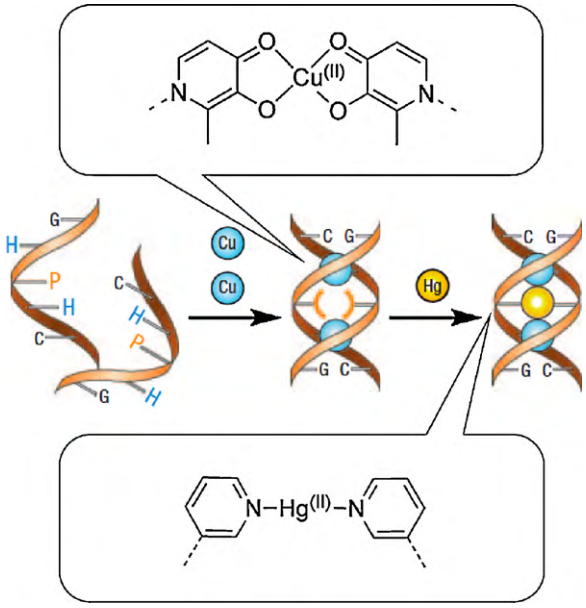

Fig. 16. Mixed metal arrays I.

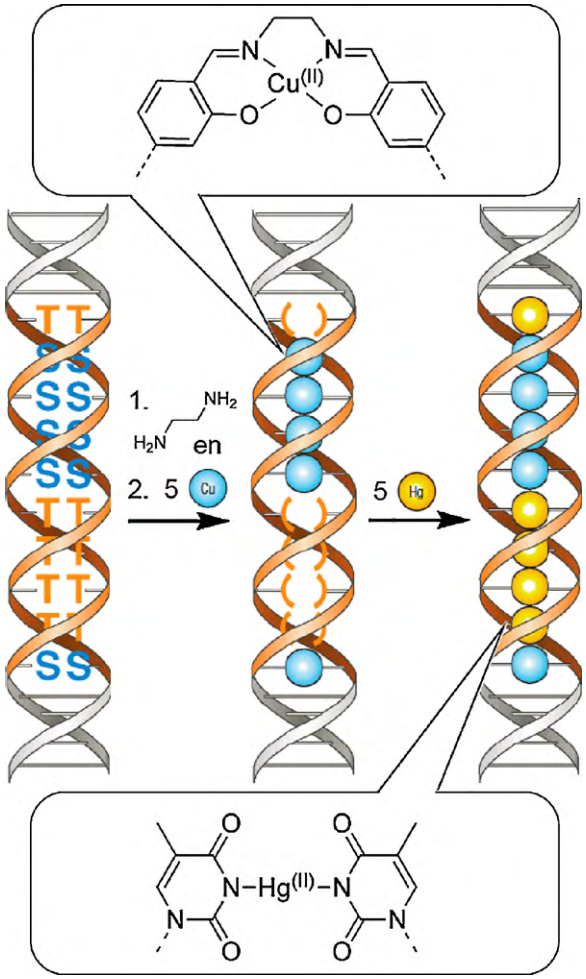

Fig. 17. Mixed metal arrays II.

spectroscopic study involving the direct measurement of the $^{1}J(^{15}N, ^{107/109}Ag)$ coupling constants between the ligands and the metal ions. According to these measurements, the duplex adopts a B-type conformation with only minor deviations in the region of the metal–base pairs [62].

After the possibility of stacking of several metal ions inside the DNA has been realized in practice, the mixing of two (or more) different metal ions inside DNA was envisioned as the next milestone. Therefore, two ligand-modified nucleosides with orthogonal coordination capabilities for two different kinds of metal ions were identified and incorporated into artificial oligonucleotides in the form of mixed arrays [63].

In a conjoint research effort, the groups around Shionoya and Carell succeeded in presenting two different combinations of two types of ligands each, which were shown to be able to bind Cu(II) and Hg(II) ions sequence-specifically in the same duplex [64]. The system presented by Shionoya et al. comprised the hydroxypyridone ligand **H** known for its ability to coordinate Cu(II) and the pyridine ligand **P** capable of coordinating Hg(II) (Fig. 16). By performing UV- and CD-spectroscopic titration experiments with consecutive addition of both metal ions to the oligonucleotide d(5′-G**HPH**C-3′) and subsequent characterization of the reaction products by ESI mass spectroscopy the formation of a duplex containing one Hg(II) and two Cu(II) ions was shown [64].

Similarly, Carell et al. synthesized strands containing a predetermined sequence of five salen ligands **S** with the propensity to bind Cu(II) and five **TT** mismatches capable of binding Hg(II) (Fig. 17). Also in this case, the incorporation of the right number of Cu(II) and Hg(II) ions according to the programmed sequence was indicated by CD-spectroscopic titration studies and the products were indentified by ESI mass spectrometry [64].

In this way, several different sequences of Cu(II) and Hg(II) ions could be aligned inside the DNA strands and thus the binary pattern that was encoded into the oligonucleotide by the automated solid-phase synthesis could be manifested into a determined array of metal ions [64]. In a similar manner, Ono and co-workers were able to incorporate **T**–Hg–**T** and **C**–Ag–**C** metal–base pairs into the same double strand [65].

4. Conclusion and future prospects

The field of metal–base pairing in DNA has developed within 10 years from the first attempts to coordinate single metal ions by monomeric ligand-modified nucleosides to rather intricate systems such as metal-mediated triple helices and programmable multi-metal stacks inside DNA duplexes [19]. The good understanding of the factors leading to stable metal–base pairing such as (1) complex stability and acidity constants, (2) geometrical prerequisites and (3) compatibility with the natural base pairs that were gathered by many interdisciplinary research groups worldwide have set a stable foundation for the application of artificial metal–base pairs in even more complex nanoscopic constructs.

From a structural point of view, the achieved duplex stabilization compared to unmodified oligonucleotides might find interesting applications in the field of DNA nano architecture by contributing to the limited binding patterns between short stretches of unmodified single-stranded DNA. Furthermore, the switching of hybridization by the absence or presence of the required metal ions (or a change in their redox state) and the discussed possibility to control structural interconversions such as hairpin to duplex transitions might lead to a progress of the mostly static DNA nano architectures to more dynamic assemblies.

From another perspective, the precise positioning of transition metal ions in a 3-dimensional space made up by the surrounding oligonucleotide framework might yield mimics of multi-metal enzymes with functions such as catalysis, charge separation or

selective guest recognition. The magnetic interaction between closely coordinated paramagnetic metal ions such as Cu(II) has already been shown to result in molecular magnetism, a topic highly interesting for information processing and storage on (a silicon-free) molecular scale.

Furthermore, the substitution of some or all natural base pairs by metal–base pairs may present great potential for the overall enhancement of charge conductivity through oligonucleotide-based materials and so open the door to an entire new area of DNA-based molecular electronics. Further synthetic modification of the ligands and a broader choice of metal ions might be necessary to fine-tune the prospect electronic functions.

Keeping in mind the various switching processes, even more sophisticated electronic functions beyond pure charge transport such as processing of logical inputs may be achieved using metal-modified DNA. First examples going into this direction are illustrated by the application of orthogonal metal–base pairing (**T**–Hg–**T** vs. **C**–Ag–**C**) in combination with the optical properties of quantum dots as sensors for Hg(II) and Ag(I) ions and as logical AND and OR gates [34].

Combined with the potential charge transport capabilities, the newly discovered magnetic properties of the metal-containing oligonucleotides may become of great importance for the emerging field of spintronics. Also an application in future quantum computing devices is imaginable.

The obtained mixed metal arrays on the one hand serve as formidable examples for the possibility to incorporate new base pairing principles orthogonal to and alongside with the natural Watson–Crick base pairs into DNA strands. On the other hand, the sequence-specific arrangement of two different kinds of metal ions might furthermore contribute to solve the problem of mispairing in long ligand-modified metal DNA complexes. Whereas it is imaginable that the formation of a perfect double strand containing e.g. 1000 consecutive metal–base pairs of the same kind might be a difficult task in terms of entropy and lead to a wild mixture of branched products, the programming of a unique sequence of two (or more) orthogonal metal–base pairs might solve this problem.

With a focus on molecular electronics, upcoming work has to unambiguously establish the conductive properties of metal-containing DNA strands e.g. by single molecule measurements in break junctions [16a], single electron transfer studies with charge injectors and traps attached to model duplexes [17] or spectroscopic methods that yield information about charge carrier availability [66]. Finally, the understanding of the electronic properties of metal-containing oligonucleotides will require the contribution from theoreticians to expand the various charge transfer models that have been developed for unmodified DNA in the recent years to include experimentally reproducible explanations for metal–base pairs in DNA.

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