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REVIEW ARTICLE

Rational design of metal-responsive functional DNA supramolecules

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Abstract
DNA molecules have excellent molecular recognition abilities through the complementary hydrogen-bonded base pairing. Since the hybridization of oligonucleotides can be programmed based on the sequences of the nucleobases, a great number of DNA supramolecular architectures have been constructed via self-assembly processes. The development of stimuli-responsive DNA supramolecules has attracted increasing interests because it will contribute to the construction of dynamic molecular systems such as molecular machines. Metal ions are considered as useful chemical stimuli, but the construction of metal-responsive DNA systems is still in the early stage. This review article describes current progress on the development of DNA supramolecules whose structure and function can be regulated in response to metal ions, with mainly focusing on our recent studies. The basic strategy is the introduction of unnatural metal ligands that form interstrand metal complexes in DNA structures. For example, artificial metal-mediated base pairs, formed through complexation between ligand-type nucleobase analogs and a bridging metal ion, were incorporated into known DNAzymes (catalytic DNA) to allosterically regulate their activity in a metal-responsive manner. Novel ligand-type nucleobases that form both metal-mediated and hydrogen-bonded base pairs have been recently devised as metal-responsive building blocks, and were used to construct a simple prototype of DNA molecular machines. Branched DNA structures bearing metal ligands at the junction core were also synthesized as novel structural motifs, with which metal-mediated structure transformation was demonstrated. These metal-responsive DNA supramolecules are expected to expand the toolbox of DNA-based supramolecular chemistry and nanotechnology.

Keywords DNA nanotechnology · Bioinorganic chemistry · Modified DNA · DNAzyme · Molecular switching · Metal coordination

Introduction

DNA molecules serve as the bearer of genetic information of living organisms, thus being recognized as one of the most important biomolecules. The DNA molecules form well-defined double helical structures through the formation of complementary Watson–Crick base pairs, A–T and G–C, with two and three internucleobase hydrogen bonds, respectively (Fig. 1a). The complementary base pairing endows DNA with exquisite molecular recognition abilities, allowing for replication and transfer of genetic information in biological systems. The molecular recognition of DNA molecules has also inspired synthetic chemists including researchers in the field of supramolecular chemistry [1]. Since the hybridization of oligonucleotides can be programmed based on the base sequences, a great number of DNA supramolecular architectures have been made from DNA molecules via self-assembly processes (Fig. 1b) [2–4]. Particularly, various two- and three-dimensional nano-sized structures can be constructed by sophisticated strategies such as DNA origami, and the computer-aided design is now available (Fig. 1c and d) [5–7]. In addition, the development of dynamic DNA molecular devices whose configuration and function can be controlled by external stimuli has attracted increasing interests. By programming the hybridization of DNA components, excellent prototypes of

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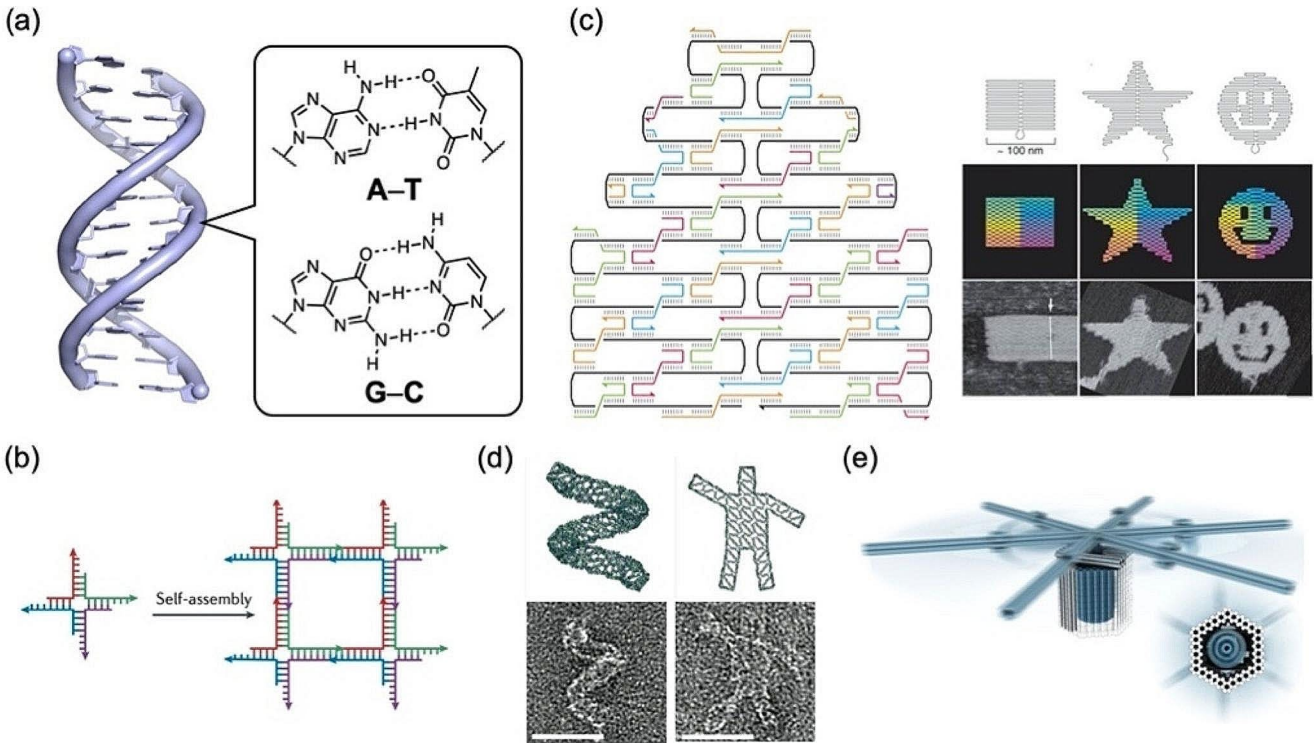

Fig. 1 **a** Schematic representation of a natural DNA duplex and the chemical structures of hydrogen-bonded Watson–Crick base pairs. **b** An example of DNA self-assembly. A DNA four-way junction structure self-assembles into a quadrilateral shape through the hybridization of the single-stranded regions called sticky ends. **c** Two-dimensional DNA origami structures [5]. Left panel: folding of a long genomic DNA strand into a designed structure with the aid of short staple strands. Right panels: atomic force microscopy images of DNA origami shapes. **d** Three-dimensional wireframe DNA origami structures imaged by negative-stain dry-state transmission electron microscopy [6]. Scale bars are 50 nm. **e** A nanoscale rotary device formed from 3D DNA components [8]. Panel **b** is adapted with permission from ref. 3. Copyright 2017 Springer Nature. Panel **c** is adapted with permission from ref. 5. Copyright 2006 Springer Nature. Panel **d** is adapted with permission from ref. 6. Copyright 2015 Springer Nature. Panel **e** is adapted from ref. 8, CC BY 4.0

molecular machines as well as computational systems have been developed thus far (Fig. 1e) [8–11].

Chemical modification of DNA molecules is a powerful way to embed stimuli-responsiveness into DNA molecules [12]. A variety of unnatural components have been incorporated into DNA oligomers based on a well-established phosphoramidite chemistry, by which nucleoside monomers are oligomerized in a stepwise manner. Post-synthetic modifications [13, 14] and enzymatic synthesis [15–17] are also useful techniques to introduce artificial functionalities into DNA. One of the successful examples is the incorporation of photo-responsive molecules, through which numerous DNA nanoarchitectures that change their shape and function in response to light irradiation were developed [18–20]. Metal ions are also considered as useful chemical stimuli by virtue of the kinetic reversibility of metal coordination bonding [21–24]. In the field of supramolecular chemistry, metal ions have been widely exploited to operate non-biological molecular switches and machines. However, the construction of metal-responsive DNA systems is still in the early stage due to the limited types of interactions between metal ions and natural DNA molecules.

One of the promising approaches to develop metal-responsive DNA supramolecules is the introduction of unnatural metal ligands into DNA [25]. The formation of interstrand metal complexes is expected to enhance the stability of the whole DNA structures, thus allowing for metal-triggered hybridization and transformation of DNAs [26]. The simplest example is the complexation between two opposing nucleobases (or nucleobase analogs) and a bridging metal ion in DNA duplexes, termed as metal-mediated base pairing (Fig. 2) [27–31]. While the natural pyrimidine nucleobases, T and C, are known to form metal-mediated-base pairs with Hg^{II} and Ag^{I} ions [32], a variety of unnatural ligand-type nucleobases that can form metal-mediated base pairs have been synthesized so far (Fig. 2a) [33–37]. Monodentate to tridentate nucleobase analogs were designed to form a 2:1 complex, i.e., a metal-mediated base pair, within DNA duplexes. In most cases, metal ions that adopt square-planar (such as Cu^{II} and Ni^{II}) or linear coordination geometry (such as Ag^{I}) are utilized so that the resulting metal-mediated base pairs are fit in very well with the stacked natural base pairs. For example, the 3-hydroxy-4-pyridone-type nucleobase (**H**) was found to form a stable

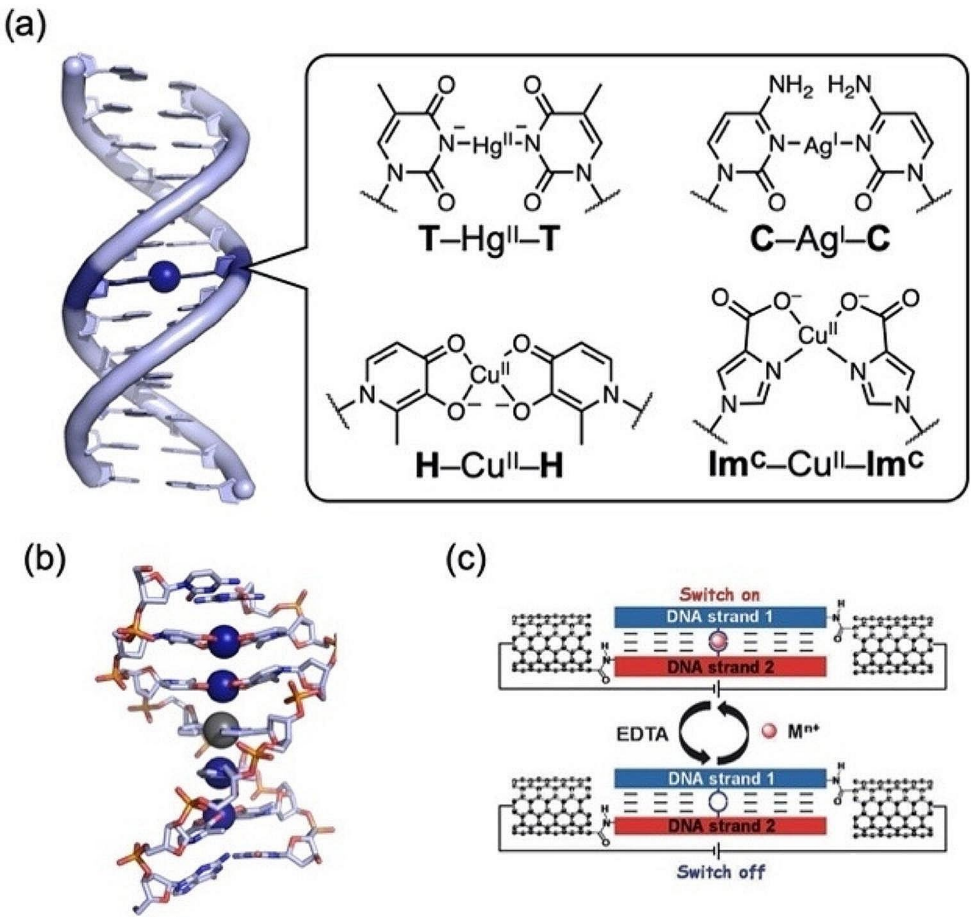

Fig. 2 **a** Schematic representation of a DNA duplex with an artificial metal-mediated base pair. Chemical structures of representative metal-mediated base pairs are shown. **b** DNA-templated heterologous metal assembly (Cu^{II}–Cu^{II}–Hg^{II}–Cu^{II}–Cu^{II}) by using two kinds of metal-mediated base pairs [41]. **c** Cu^{II}-dependent regulation of the conductivity of metallo-DNA devices filling gaps in carbon nanotubes [43]. Panel **c** is reproduced with permission form ref. 43. Copyright 2011 Wiley-VCH

Cu^{II}-mediated base pair (**H**–Cu^{II}–**H**) inside DNA duplexes [38]. The metal-mediated base pairs have originally been applied to the construction of one-dimensional metal arrays (Fig. 2b) [39–42], and the physical properties of metallo-DNA structures were then investigated (Fig. 2c) [40, 43]. The T–Hg^{II}–T and C–Ag^{I}–C base pairs have been also used to create Hg^{II}- or Ag^{I}-responsive DNA molecular devices [32], but unintended binding of these metal ions to other T and C bases causes difficulties in the molecular design. Therefore, it was highly desired to use other metal-mediated base pairs consisting of ligand-type nucleobase analogs and metal ions other than Hg^{II} or Ag^{I} for the rational design of metal-responsive DNA systems.

This review describes current progress on the development of DNA supramolecules whose structure and function can be regulated in response to metal ions, with mainly focusing on our recent studies. The basic strategy is the introduction of unnatural metal ligands that form interstrand metal complexes in DNA structures. For example, the incorporation of artificial metal-mediated base pairs into known DNAzymes (catalytic DNA) allows for allosteric regulation of their catalytic activity in a metal-responsive manner. Novel ligand-type nucleobases that form both metal-mediated and hydrogen-bonded base pairs have been devised as metal-responsive building blocks, and were used to construct a simple prototype of DNA molecular machines. Branched DNA structures bearing metal ligands at the junction core were also synthesized as novel structural motifs, with which metal-mediated structure transformation was demonstrated. These metal-responsive DNA supramolecules are expected to expand the toolbox of DNA-based supramolecular chemistry and nanotechnology. The design strategy of each metal-responsive DNA supramolecule will be discussed in detail in the following sections.

Rational deign of metal-responsive split DNAzymes

Among various functional nucleic acids, DNAzymes (deoxyribozymes) have attracted much attention because of their unique property to catalyze chemical reactions [44]. DNAzymes are normally obtained through a process called in vitro selection or SELEX (Systematic Evolution of Ligands by EXponential enrichment), during which catalytically active DNA oligomers are selected from DNA

strands with randomized base sequences. Especially, DNAzymes that catalyze the cleavage of nucleic acid substrates have been widely utilized to develop DNA-based molecular sensors, molecular machines, and molecular computing systems [45–47]. In such applications, the rational design of stimuli-responsive allosteric DNAzymes is thought to be an important challenge [48]. So far, DNAzymes that are activated by the addition of target DNA/RNA [49] and small molecules [50] and by photoirradiation [51] were developed using unmodified or chemically-modified oligonucleotides. Metal-responsive DNAzymes consisting of only natural nucleotides were previously developed based on the T–Hg^{II}–T and C–Ag^{I}–C base pairing by Willner, Lu, and others (Fig. 3) [52, 53]. However, the metal ions used as stimuli were limited to only Hg^{II} and Ag^{I} ions, which strongly interact with natural nucleobases.

We have developed a series of metal-responsive DNAzymes whose catalytic activity can be regulated in response to metal ions other than Hg^{II} and Ag^{I}, by using artificial metal-mediated base pairs (Fig. 4). The base sequences were strategically designed based on previously-identified DNAzyme sequences so that the modified DNAzymes are activated through the formation of metal-mediated base pairs. One of the simplest strategies is to split the parent DNAzymes into two fragments and introduce a metal-mediated base pair(s) into the stem duplex (Fig. 4b). The formation of the metal-mediated base pair was expected to induce hybridization of the split strands, resulting in metal-dependent reconstruction of the active DNAzyme structure.

This design strategy was initially used to reshape a well-studied RNA-cleaving DNAzyme called E5 [54] into a Cu^{II}-responsive DNAzyme [55]. The E5 DNAzyme (Fig. 4a), composed of a catalytic core, a substrate-binding domain, and a variable stem, was segmented at the loop region, and a pair of **H** nucleotides [38] were incorporated into the stem duplex. The **H**-modified split DNAzyme was expected to lose its activity and to restore its catalytically active structure in the presence of Cu^{II} ions via **H**–Cu^{II}–**H** base pairing. The length of the stem duplex and the position of the **H** nucleotides were varied to determine the optimal molecular design. When an **H**–**H** pair was introduced near the catalytic core, the modified DNAzyme exhibited a very low activity even in the presence of Cu^{II} ions. This is presumably due to the structural distortion of the catalytic core arising from the larger size of the **H**–Cu^{II}–**H** pair (whose C1′–C1′ distance was estimated to be 12.7 Å in a DNA analog [56]) compared to the natural Watson–Crick base pairs (10.7 Å). When an **H**–**H** mismatch was located at the terminal of the stem duplex, the activity of the DNAzyme was little changed by the addition of Cu^{II} ions. This is likely because the duplex with an **H**–**H** pair at the end is stable enough to be associated even without Cu^{II} ions. Among the sequences tested, a split DNAzyme with an 8-base-pair stem possessing an **H**–**H** pair at the third position from the catalytic core was found to exhibit the best response to Cu^{II} ions. The base sequence of the optimized **H**-modified DNAzyme (named as **H-Dz1**) is shown in Fig. 4c.

The activity of the resulting **H-Dz1** can be estimated by analyzing the time-course of the RNA-cleaving reactions

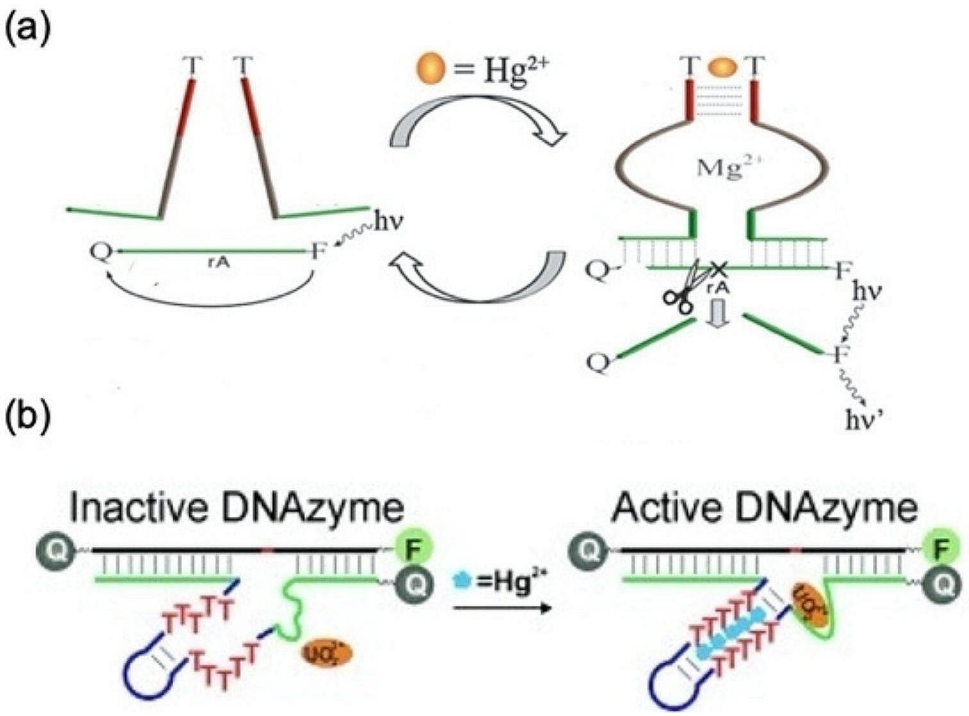

Fig. 3 Hg^{II}-responsive DNAzymes based on T–Hg^{II}–T base pairing. **a** Hg^{II}-responsive split DNAzyme reported by Willner et al. [52]. **b** Hg^{II}-responsive single-stranded DNAzyme reported by Lu et al. [53]. Panel **a** is adapted with permission from ref. 52. Copyright 2010 Royal Society of Chemistry. Panel **b** is adapted with permission form ref. 53. Copyright 2007 Wiley-VCH

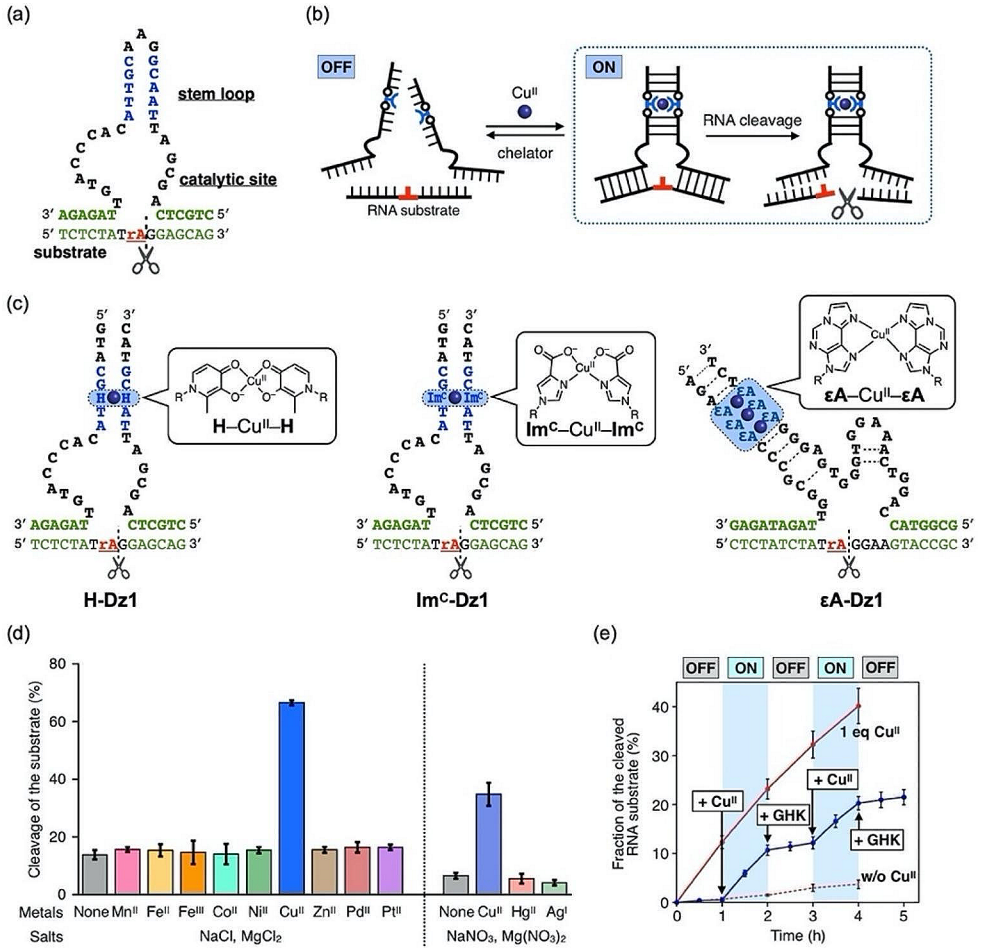

Fig. 4 Development of metal-responsive split DNAzymes by using metal-mediated base pairing. **a** Base sequences of RNA-cleaving E5 DNAzyme consisting of natural nucleotides. "rA" in the substrate strand represents an adenine ribonucleotide at the cleavage site. **b** Design principle of metal-responsive split DNAzymes. **c** Base sequences of Cu^{II}-responsive split DNAzymes with metal-mediated base pairs. **d** The RNA-cleaving activity of **H-Dz1** in the presence of various transition metal ions. [DNAzyme] = 1.0 μM, [substrate] = 1.0 μM, [metal ions] = 1.0 μM (1.0 equiv) in 10 mM HEPES (pH 7.0) containing 1 M NaCl and 10 mM $MgCl_2$, 25 °C, 3 h. $N = 3$. For the experiments with Hg^{II} and Ag^{I} ions, NaCl and $MgCl_2$ in the buffer were replaced with $NaNO_3$ and $Mg(NO_3)_2$, respectively. Error bars indicate standard errors. **e** Iterative switching of the activity of **Im^C-Dz1**. Cu^{II} (1 equiv), GHK (2 equiv), Cu^{II} (2 equiv), and GHK (4 equiv) were alternately added. [DNAzyme] = 1.0 μM, [substrate] = 10 μM (10 equiv), 25 °C. $N = 3$. The DNAzyme activity in the presence (red solid lines) and the absence of Cu^{II} ions (red dotted lines) are also shown. Error bars indicate standard errors. Panels **b** and **d** are reproduced with permission form ref. 55. Copyright 2019 American Chemical Society. Panel **e** is reproduced with permission form ref. 58. Copyright 2020 Wiley-VCH

in the absence and the presence of Cu^{II} ions. Under the Cu^{II}-free conditions, the RNA-cleaving activity of **H-Dz1** was significantly lower (apparent first-order rate constant k_{obs} = 0.029 h^{-1}) than that of the original E5 DNAzyme (0.23 h^{-1}). When one equivalent of Cu^{II} ions was added, the DNAzyme activity was enhanced 5.5-fold (k_{obs} = 0.16 h^{-1}). In contrast, the activity of the unmodified E5 DNAzyme was not increased upon Cu^{II} addition. Under the same reaction conditions, Cu^{II} ions cannot cleave the RNA substate without the DNAzyme strands. Therefore, it was concluded that the addition of Cu^{II} ions increased the catalytic activity of **H-Dz1**. Additionally, titration experiments confirmed that one equivalent of Cu^{II} ions is sufficient to activate **H-Dz1**. All of these results agree well with the action mechanism that the formation of an **H**–Cu^{II}–**H** base pair induces the hybridization of the split strands to restore the active DNAzyme structure. Important to note is that the addition of other transition metal ions such as Mn^{II}, Fe^{II}, Fe^{III}, Co^{II}, Ni^{II}, Zn^{II}, Pd^{II}, Pt^{II}, Hg^{II}, and Ag^{I} did not activate **H-Dz1**, showing a good metal selectivity (Fig. 4d). This result coincides with the metal specificity of the **H**–Cu^{II}–**H** base pairing. It was also demonstrated that the activity of **H-Dz1** can be reversibly regulated by the addition and the removal of Cu^{II} ions. Sequential addition of equimolar amounts of Cu^{II} ions and a Cu^{II}-binding peptide (GHK) [57] resulted in the iterative switching of the DNAzyme activity. Furthermore, **H-Dz1**

was found to be inactivated and re-activated in responce to the redox of Cu^{II} ions.

More efficient on-off switching was demonstrated with a split DNAzyme containing a different Cu^{II}-mediated base pair $\mathbf{Im^C}$–Cu^{II}–$\mathbf{Im^C}$ (Fig. 4c) [58], which shows a greater duplex stabilization effect ($\Delta T_m = +35.2$ °C) than the **H**–Cu^{II}–**H** base pair (+13.1 °C) [58, 59]. The $\mathbf{Im^C}$-modified split DNAzyme (**Im^C-Dz1**) was designed by formally replacing the **H** nucleotides in **H-Dz1** with $\mathbf{Im^C}$ nucleotides. In the absence of Cu^{II} ions, the RNA-cleaving activity of **Im^C-Dz1** was greatly suppressed. This is because the two fragments of the split DNAzyme was separated by the electric repulsion between the negatively charged $\mathbf{Im^C}$ bases in the stem duplex. The addition of equimolar Cu^{II} ions enhanced the DNAzyme activity ca. 12-fold through the re-association of the split DNAzyme by $\mathbf{Im^C}$–Cu^{II}–$\mathbf{Im^C}$ base pairing. These results confirmed that **Im^C-Dz1** was activated in a Cu^{II}-responsive manner as is the case for **H-Dz1**, and the on-off ratio was improved by changing the ligand-type nucleobases. Additionally, the activity of **Im^C-Dz1** was shown to be reversibly regulated by addition, removal, and reduction of Cu^{II} ions during the reaction. As shown in Fig. 4e, the alternate addition of Cu^{II} and GHK peptide resulted in repetitive switching of the DNAzyme activity.

Metal-responsive DNAzymes were further developed by using damaged nucleobases as metal-binding sites (Fig. 4c) [60]. We employed a 1,N^6-ethenoadenine (**εA**), an etheno adduct of a natural adenine base, because it was reported to form Cu^{II}-mediated **εA**–Cu^{II}–**εA** base pairs in DNA duplexes [61]. In a manner similar to the abovementioned split DNAzymes, an RNA-cleaving DNAzyme named NaA43 [62] was split into two fragments. Three **εA**–**εA** mismatch pairs were consecutively introduced into the stem duplex because our investigations revealed that the formation of at least three **εA**–Cu^{II}–**εA** base pairs is needed for substantial stabilization of DNA duplexes. As a result, the activity of the **εA**-modified split DNAzyme (**εA-Dz1**) was shown to be enhanced 5.3-fold by the addition of three equivalents of Cu^{II} ions (i.e., $[Cu^{II}]/[\mathbf{εA}–\mathbf{εA}] = 1.0$).

Rational deign of metal-responsive single-stranded allosteric DNAzymes

Metal-responsive allosteric DNAzymes can be rationally designed without splitting the original DNAzymes (Fig. 5) [63]. This strategy involves intrastrand structural transformation caused by metal-mediated base pairing (Fig. 5a). A metal-mediated base pair(s) is incorporated into the stem duplex of the parent DNAzyme in a manner similar to the split DNAzymes discussed above. Additionally, the loop sequence is altered to hybridize with the catalytic core to

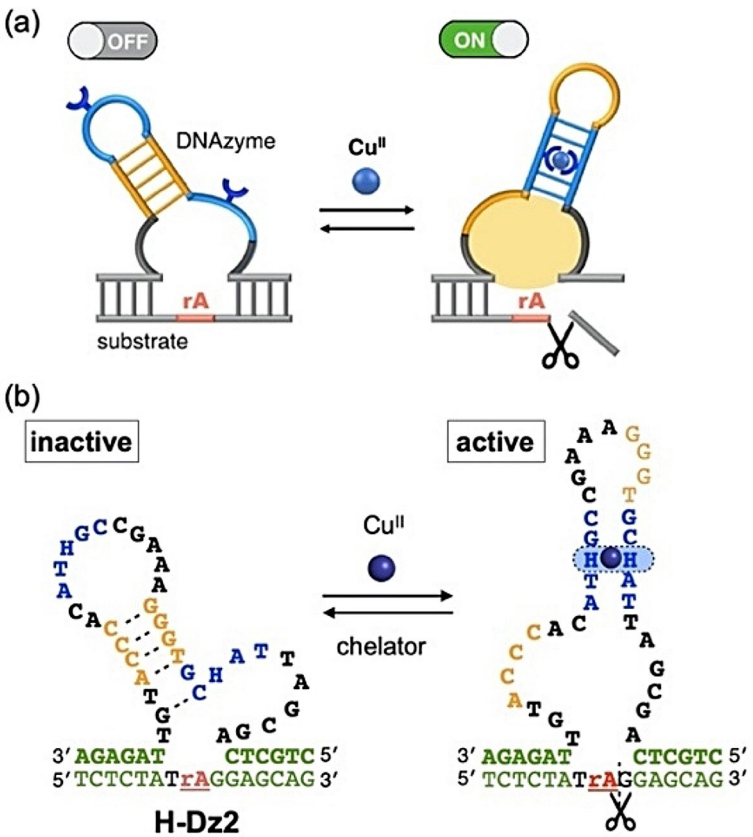

Fig. 5 Development of metal-responsive single-stranded allosteric DNAzymes by using metal-mediated base pairs. **a** Schematic representation of a Cu^{II}-responsive single-stranded DNAzyme. **b** Base sequences of a Cu^{II}-responsive DNAzyme with an **H**–Cu^{II}–**H** base pair (**H-Dz2**). Both catalytically inactive and active secondary structures are shown. "rA" in the substrate strand represents an adenine ribonucleotide at the cleavage site. Panel **a** is reproduced with permission form ref. 63. Copyright 2020 American Chemical Society

decrease the DNAzyme activity in the absence of the target metal ions. The addition of the metal ions is expected to trigger structural changes from a catalytically inactive structure to an active DNAzyme structure with metal-mediated base pair(s). For example, the RNA-cleaving E5 DNAzyme was reshaped to a Cu^{II}-responsive allosteric DNAzyme by the introduction of an **H**–Cu^{II}–**H** base pair (Fig. 5b) [63]. An **H**–**H** mismatch pair was incorporated into the stem region and a sequence complementary to the catalytic domain was inserted into the loop (shown in orange) to form the inactive structure. The RNA-cleaving activity of the resulting DNAzyme (**H-Dz2**) was efficiently suppressed (k_{obs} = 0.011 h^{-1}) in the absence of Cu^{II} ions as expected. The addition of Cu^{II} ions increased the DNAzyme activity by 6.8-fold (k_{obs} = 0.073 h^{-1}), showing better on-off performance than the split DNAzyme **H-Dz1**. A fluorescence assay using a DNAzyme modified with a fluorogenic nucleobase analog (pyrrolocytosine) [64] confirmed the intrastrand structure conversion induced by the addition of Cu^{II}. Titration of Cu^{II} ions established that one equivalent of Cu^{II} ions is sufficient to activate **H-Dz2**. These results showed that the non-split **H-Dz2** was allosterically regulated through the quantitative formation of an **H**–Cu^{II}–**H** base pair. Furthermore, reversible control of the DNAzyme activity was demonstrated by the addition and the removal of Cu^{II} ions under isothermal conditions. Alternate addition of Cu^{II} ions and a Cu^{II}-binding peptide (GHK) resulted in a repetitive switching of the DNAzyme activity.

We subsequently developed a Cu^{II}-responsive allosteric DNAzyme containing multiple metal-mediated base pairs [65]. Three **H**–Cu^{II}–**H** base pairs were consecutively introduced into the parent E5 DNAzyme and the loop sequence was modified as is the case for **H-Dz2**. The activity of the resulting DNAzyme (**H-Dz3**) was found to increase 2.2-fold by the addition of three equivalents of Cu^{II} ions (i.e., [Cu^{II}]/[**H**–**H** mismatch pair] = 1.0). Contrary to our expectations, the on-off ratio was lower than that of **H-Dz2** containing only a single **H**–Cu^{II}–**H** pair. It is likely that the structural distortion caused by multiple **H**–Cu^{II}–**H** base pairs adversely influenced on the DNAzyme activity.

The design strategy of the metal-responsive non-split DNAzymes was further applied to other types of DNAzymes such as an RNA-cleaving NaA43 DNAzyme [63]. A pair of **H** nucleotides were incorporated into the stem as a Cu^{II}-binding site and the stem duplex was shortened to form a catalytically inactive structure in the absence of Cu^{II} ions. The activity of the modified DNAzyme (**H-Dz4**) was enhanced 5.9-fold upon addition of equimolar Cu^{II} ions, demonstrating the allosteric regulation of the DNAzyme activity in response to Cu^{II}. Recently, we have remade an RNA-cleaving 8–17 DNAzyme to a Cu^{II}-responsive allosteric DNAzyme (Fig. 6) [66]. The 8–17 DNAzyme is one of the smallest DNAzymes and does not have any long stem region (Fig. 6a) [67]. Owing to its compactly-folded structure, it has been challenging to modify 8–17 DNAzyme without losing its activity. Based on the reported crystal structure [68], an $\mathbf{Im^C}$–Cu^{II}–$\mathbf{Im^C}$ base pair was introduced in the middle of the 3-base-pair duplex region inside the pseudoknot structure (Fig. 6c). It was expected that the modified DNAzyme ($\mathbf{Im^C}$**-Dz2**) is folded into the catalytically active form through the formation of the $\mathbf{Im^C}$–Cu^{II}–$\mathbf{Im^C}$ base pair. As a result, the activity of $\mathbf{Im^C}$**-Dz2** was enhanced 5.1-fold upon addition of one equivalent of Cu^{II} ions, showing good metal responsiveness. The development of the compactly-packed Cu^{II}-responsive DNAzyme ($\mathbf{Im^C}$**-Dz2**) demonstrates the utility of metal-mediated artificial base pairs for creating stimuli-responsive functional DNAs.

The same approach was applied to the development of a DNAzyme that exhibits an AND logic-gate response to two different metal ions, Cu^{II} and Ag^{I} (Fig. 7) [63]. An

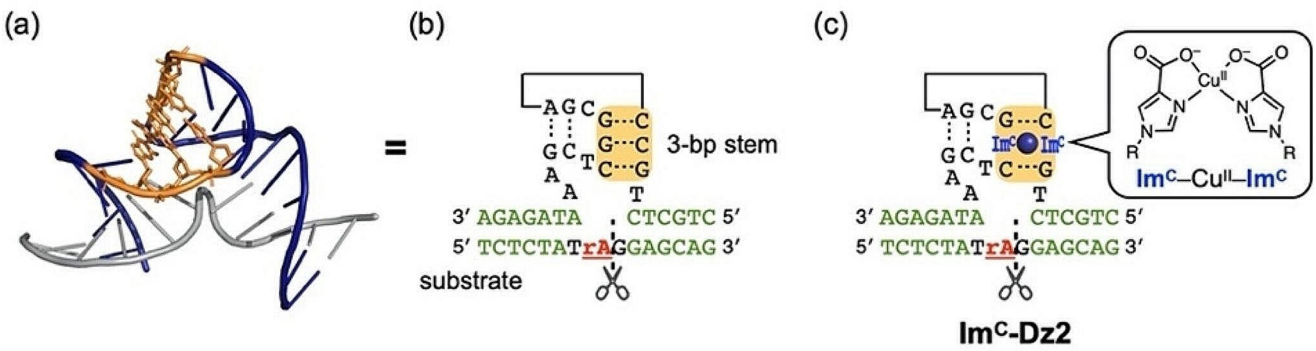

Fig. 6 Development of Cu^{II}-responsive 8–17 DNAzyme. **a** Crystal structure of a compact-sized 8–17 DNAzyme reported previously (PDB: 5XM8). The DNAzyme and the substrate are depicted in blue and grey, respectively. The 3-base-pair stem is colored in orange. **b** Base sequence of the unmodified 8–17 DNAzyme. The broken lines indicate hydrogen-bonded base pairs (including a nonstandard A–G base pair) to form the pseudoknot structure. **c** Molecular design of Cu^{II}-responsive 8–17 DNAzyme containing an $\mathbf{Im^C}$–Cu^{II}–$\mathbf{Im^C}$ base pair ($\mathbf{Im^C}$**-Dz2**). "rA" represents an adenine ribonucleotide at the cleavage site. Reprinted with permission from ref. 66. Copyright 2024 Royal Society of Chemistry

Ag^{I}-dependent RNA-cleaving DNAzyme Ag10c, previously obtained by SELEX [69], was modified to be activated in the presence of Cu^{II} ions. An **H**–**H** mismatch pair was incorporated in the stem region and the loop sequence was changed to form inactive structure under Cu^{II}-free conditions (Fig. 7a). The catalytic activity of the modified DNAzyme (**H-Dz5**) was tested in the absence and the presence of Cu^{II} (1 equiv) and Ag^{I} ions (10 equiv) (Fig. 7b). In the absence of Ag^{I} ions, the DNAzyme showed no catalytic activity at all. In the presence of only Ag^{I} ions but without Cu^{II}, the DNAzyme showed only faint activity. When both Cu^{II} and Ag^{I} ions were added, the DNAzyme efficiently cleaved the RNA substrate. These results confirmed that **H-Dz5** functions as an AND logic-gate system that responds to Cu^{II} and Ag^{I} ions as inputs.

Collectively, it was demonstrated that various known DNAzymes can be reshaped into metal-responsive allosteric DNAzymes by introducing metal-mediated artificial base pairs. Although many metal-dependent DNAzymes have been obtained via SELEX method [70], chemical modification of existing DNAzymes would be a more versatile strategy to develop metal-responsive DNAzymes. In our approaches, one or a few pairs of ligand-type nucleobase analogs are introduced as the allosteric site. Since only minimal chemical modification is required, the design strategies described above are expected to be applied to the allosteric regulation of other functional DNA molecules. Recently, Müller et al. have developed metal-responsive DNA aptamers whose binding affinity to the target molecules can be regulated by the addition of metal ions [71, 72]. Ligand-type nucleotides, such as **Im** and $\mathbf{Im^{C}}$, were incorporated into known aptamer sequences so that the active structure can be reconstituted through the formation of metal-meditated base pairs, such as **Im**–Ag^{I}–**Im** [73] and $\mathbf{Im^{C}}$–Cu^{II}–$\mathbf{Im^{C}}$ [59]. It is also expected that allosteric DNA molecules responsive to different effector metal ions can be developed by using appropriate metal-mediated base pairs. Designing allosteric DNAs that are regulated by biologically important metal ions and by redox of metal ions would be of significant interest in terms of future applications in biological systems [74, 75]. Furthermore, the use of metal ions that little interfere with natural nucleobases would allow for the applications of the metal-responsive DNAs in more complex DNA systems including DNA nanomachines and DNA computing systems.

Development of metal-responsive bifacial nucleobases

As discussed above, artificial metal-mediated base pairing is a versatile tool to create metal-responsive DNA supramolecules. In order to realize more efficient switching of DNA structures and functions in response to metal ions, we have developed bifacial nucleobases that form both hydrogen-bonded and metal-mediated base pairs (Fig. 8). The bifacial nucleobases were designed so that their base-pairing partner can be altered by the addition of specific metal ions. In particular, the bifacial nucleobases have a Watson–Crick face and an additional metal-coordination site on the other side (Fig. 8a). We first examined a 5-hydroxyuracil ($\mathbf{U^{OH}}$) base,

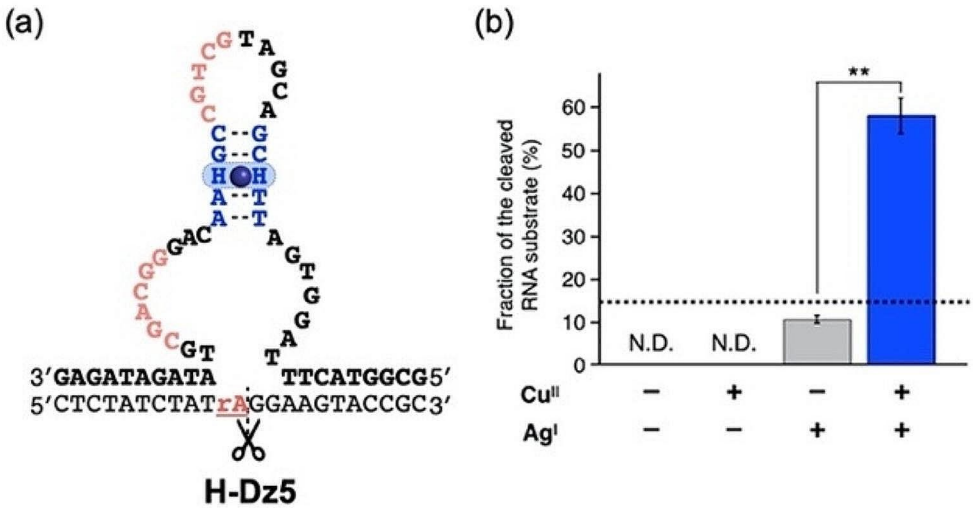

Fig. 7 Development of an AND-gate DNAzyme that responds to Cu^{II} and Ag^{I} ions. **a** Base sequence of the **H**-modified AND-gate DNAzyme (**H-Dz5**). Only the active structure is shown. The sequence shown in orange is complementary to each other, allowing for the formation of inactive structure in the absence of Cu^{II}. "rA" in the substrate represents adenine ribonucleotide at the cleavage site. **b** RNA-cleaving activity of **H-Dz5** in the presence of Cu^{II} and/or Ag^{I} ions. [DNAzyme] = 1.0 μM, [substrate] = 10 μM, [$CuSO_4$] = 0 or 1.0 μM, [$AgNO_3$] = 0 or 10 μM in 10 mM HEPES buffer (pH 7.5) containing 200 mM $NaNO_3$, 25 °C, 1 h, $N = 3$. Error bars indicate standard errors. ** $p < 0.01$ (two-tailed unpaired Student's t test). Panel **b** is reproduced with permission form ref. 63. Copyright 2020 American Chemical Society

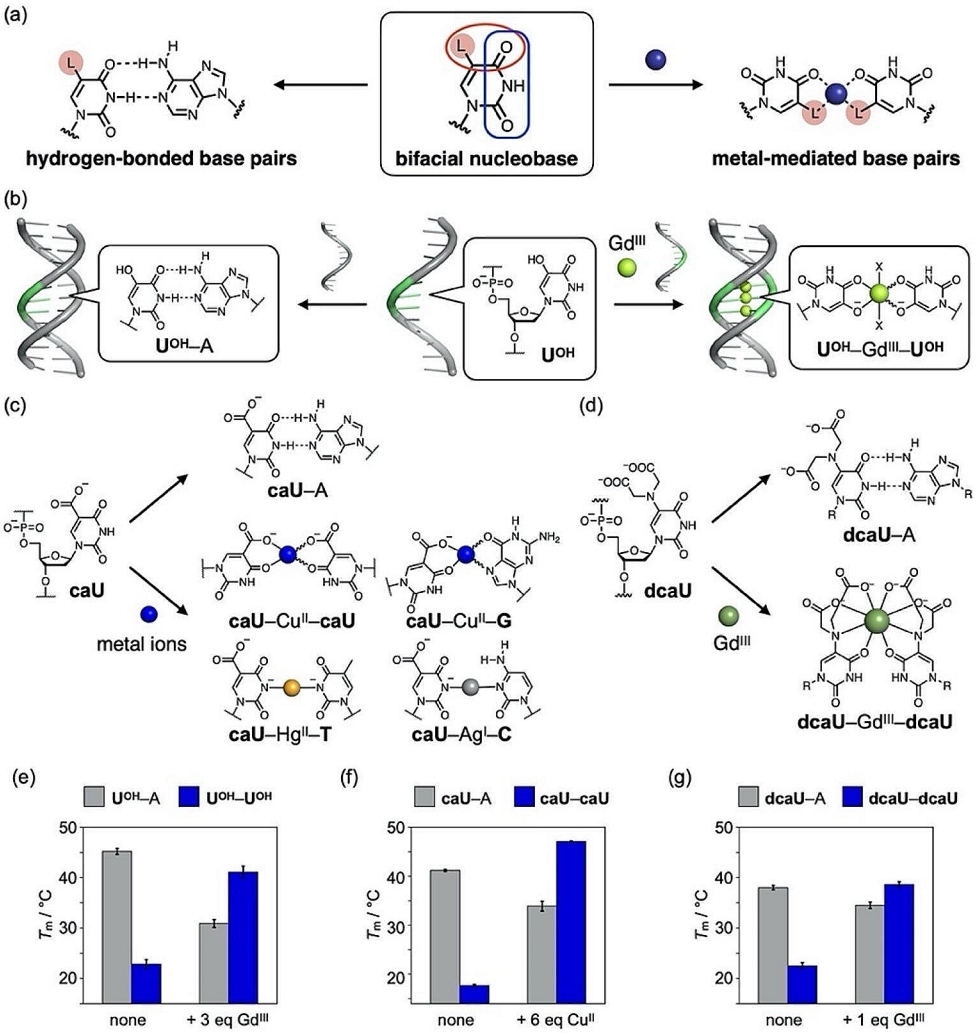

Fig. 8 Hydrogen-bonded and metal-mediated base pairing of bifacial 5-modified pyrimidine nucleobases. **a** Concept of the metal-mediated base-pair switching of the bifacial nucleobases. "L" represents a metal coordinating functionality. **b** 5-Hydroxyuracil (**U**$^{\mathbf{OH}}$) nucleobase. "X" represents additional coordinating ligands such as water molecules and adjacent nucleobases. **c** 5-Carboxyuracil (**caU**) nucleobase. **d***N*,*N*- Dicarboxymethyl-5-aminouracil (**dcaU**) nucleobase. **e** Melting temperatures (T_m) of 15-base-pair DNA duplexes containing three **U**$^{\mathbf{OH}}$–A or **U**$^{\mathbf{OH}}$–**U**$^{\mathbf{OH}}$ base pairs in the absence and the presence of Gd^{III} ions (3 equiv). **f** T_m values of 15-base-pair DNA duplexes containing three **caU**–A or **caU**–**caU** base pairs in the absence and the presence of Cu^{II} ions (6 equiv). **g** T_m values of 15-base-pair DNA duplexes containing a **dcaU**–A or **dcaU**–**dcaU** base pair in the absence and the presence of Gd^{III} ions (1 equiv). [Duplex] = 2.0 μM in 10 mM HEPES buffer (pH 8.0 for **e**, pH 7.0 for **g** and **f**) containing 100 mM NaCl

whose 4-carbonyl and 5-hydroxy groups were considered to serve as a bidentate ligand (Fig. 8b) [76]. It was confirmed that the **U**$^{\mathbf{OH}}$ bases form Watson–Crick-type hydrogen-bonded base pair (**U**$^{\mathbf{OH}}$–A) with natural adenine (A) bases as is the case for natural thymine (T) and uracil (U) bases. In addition, the **U**$^{\mathbf{OH}}$ bases were found to form metal-mediated homo base pairs (**U**$^{\mathbf{OH}}$–M–**U**$^{\mathbf{OH}}$) in the presence of most lanthanoid ions (M = Gd^{III} etc.). The formation of metal-mediated base pairs was evidenced by DNA duplex melting analysis, UV titration experiments, and mass spectrometry. It should be noted that the stable formation of **U**$^{\mathbf{OH}}$–M–**U**$^{\mathbf{OH}}$ base pairs was observed when DNA duplexes contain at least three consecutive **U**$^{\mathbf{OH}}$–**U**$^{\mathbf{OH}}$ pairs. Since lanthanoid ions generally adopt high coordination numbers, such as 8 or 9, it is most likely that the metal centers are coordinated not only by the two opposing **U**$^{\mathbf{OH}}$ nucleobases but also by additional ligands such as water molecules and adjacent nucleobases. Although the detailed coordination structure is still unrevealed, both terminal regions of the metallo-DNA duplex were shown to adopt a typical right-handed B-form helix as indicated by circular dichroism (CD) spectroscopy and NMR structure analysis. Furthermore, **U**$^{\mathbf{OH}}$ bases were found to form Zn^{II}-mediated **U**$^{\mathbf{OH}}$–Zn^{II}–**U**$^{\mathbf{OH}}$ base pairs within DNA duplexes under basic conditions [77]. The pH-dependency can be explained by the need for the deprotonation of the 5-hydroxy group of **U**$^{\mathbf{OH}}$ ($pK_a = 7.7$).

DNA duplexes containing bifacial nucleobases such as **U**$^{\mathbf{OH}}$ showed unique thermal denaturing behaviors in the presence of the corresponding metal ions (Fig. 8e). The thermal stability of a 15-base-pair DNA duplex containing three **U**$^{\mathbf{OH}}$–**U**$^{\mathbf{OH}}$ pairs, (5′-CAC ATT **U**$^{\mathbf{OH}}$**U**$^{\mathbf{OH}}$**U**$^{\mathbf{OH}}$ GTT GTA-3′)·(3′-GTG TAA **U**$^{\mathbf{OH}}$**U**$^{\mathbf{OH}}$**U**$^{\mathbf{OH}}$ CAA CAT-5′), was greatly increased upon addition of 3 equiv of Gd^{III} ions ($\Delta T_m = +18.3$ °C). The duplex stabilization is ascribed to the additional cross-linkage by the formation of three **U**$^{\mathbf{OH}}$–Gd^{III}–**U**$^{\mathbf{OH}}$ base pairs. Interestingly, a DNA duplex containing three hydrogen-bonded **U**$^{\mathbf{OH}}$–**A** pairs, (5′-CAC ATT **U**$^{\mathbf{OH}}$**U**$^{\mathbf{OH}}$**U**$^{\mathbf{OH}}$ GTT GTA-3′)·(3′-GTG TAA **AAA** CAA CAT-5′) was destabilized by the addition of 3 equiv of Gd^{III} ($\Delta T_m = -14.3$ °C). The Gd^{III}-dependent destabilization was

caused by the binding of Gd^{III} ions to the $\mathbf{U^{OH}}$ bases, which could weaken the hydrogen-bonding between $\mathbf{U^{OH}}$ and A. Taken together, the duplex containing $\mathbf{U^{OH}}$–A pairs (T_m = 45.2 °C) is more stable than the duplex with $\mathbf{U^{OH}}$–$\mathbf{U^{OH}}$ pairs (22.8 °C) without Gd^{III} ions, and the duplex with $\mathbf{U^{OH}}$–Gd^{III}–$\mathbf{U^{OH}}$ pairs (41.1 °C) becomes more stable than the duplex with $\mathbf{U^{OH}}$–A pairs (30.9 °C) upon Gd^{III} addition (Fig. 8e). These results suggest that the hybridization preference of the $\mathbf{U^{OH}}$-containing strand, 5′-CAC ATT $\mathbf{U^{OH}U^{OH}U^{OH}}$ GTT GTA-3′, can be altered by the addition of Gd^{III} ions.

The second example of the metal-responsive bifacial nucleobases is 5-carboxyuracil (**caU**), which has a 4-carbonyl and a 5-carboxy groups as a bidentate metal binding site (Fig. 8c) [78]. The **caU** bases form not only hydrogen-bonded **caU**–A base pairs but also Cu^{II}-mediated **caU**–Cu^{II}–**caU** base pairs inside DNA duplexes. The base-pairing property of **caU** was elucidated by duplex melting analysis, CD spectroscopy, and mass spectrometry. It is interesting to note that **caU** forms metal-mediated base pair with other natural nucleobases, T, C, and G. For example, **caU**–Hg^{II}–T and **caU**–Ag^{I}–C base pairs were found to form in the presence of Hg^{II} and Ag^{I} ions, respectively, through N3 coordination in a manner similar to well-studied T–Hg^{II}–T and C–Ag^{I}–C base pairs [32]. Furthermore, the **caU** bases were suggested to pair with G bases in the presence of Cu^{II} ions to afford **caU**–Cu^{II}–G base pairs. Control experiments using 7-deazaguanines instead of G bases indicated its [*N*,*O*,*O*,*O*]-type coordination geometry. Overall, **caU** bases were shown to form a homo base pair with itself (**caU**–Cu^{II}–**caU**) and hetero base pairs with all four canonical nucleobases (**caU**–A, **caU**–Hg^{II}–T, **caU**–Ag^{I}–C, and **caU**–Cu^{II}–G) depending on the coexisting metal ions.

As is the case with $\mathbf{U^{OH}}$ bases, metal-dependent stabilization and destabilization were observed with DNA duplexes containing **caU** nucleobases (Fig. 8f). A duplex containing three consecutive **caU**–**caU** pairs, (5′-CAC ATT **caUcaU**-**caU** GTT GTA-3′)·(3′-GTG TAA **caUcaUcaU** CAA CAT-5′), was significantly stabilized by adding 3 equiv of Cu^{II} ions (ΔT_m = +30.7 °C). The Cu^{II}-mediated duplex stabilization was ascribable to the quantitative formation of three **caU**–Cu^{II}–**caU** base pairs. Importantly, the degree of the stabilization caused by **caU**–Cu^{II}–**caU** base pairing is higher than that observed with $\mathbf{U^{OH}}$–Gd^{III}–$\mathbf{U^{OH}}$ base pairing. In contrast, a duplex containing three hydrogen-bonded **caU**–**A** pairs, (5′-CAC ATT **caUcaUcaU** GTT GTA-3′)·(3′-GTG TAA **AAA** CAA CAT-5′), was destabilized when excess Cu^{II} ions (6 equiv) were added (ΔT_m = −7.3 °C). In other words, the duplex containing three **caU**–A pairs (T_m = 41.2 °C) is more stable than the duplex with three **caU**–**caU** mismatch pairs (17.7 °C) in the absence of Cu^{II} ions (Fig. 8f). In contrast, the duplex with **caU**–**caU** (47.0 °C) shows higher stability than the duplex with **caU**–A (33.9 °C) in the presence of Cu^{II} ions (6 equiv). As a result, the addition of Cu^{II} ions reversed the order of the stability of the duplexes, indicating that the hybridization preference of **caU**-containing DNA strands can be changed in response to Cu^{II} ions.

The metal-mediated duplex stabilization was observed only when multiple $\mathbf{U^{OH}}$–$\mathbf{U^{OH}}$ or **caU**–**caU** pairs are incorporated, thus limiting the flexibility of the sequence design. We have recently synthesized a novel metal-responsive bifacial nucleobase, *N*,*N*-dicarboxymethyl-5-aminouracil (**dcaU**), which possesses a tridentate iminodiacetic acid (IDA) ligand at the 5-position of the uracil base (Fig. 8d) [79]. Since the IDA ligand is a partial structure of classical chelating reagents such as EDTA, the **dcaU** base was expected to form stable complexes with various metal ions. The thermal stability of a 15-base-pair DNA duplex with a **dcaU**–**dcaU** pair in the middle, (5′-CAC ATT A**dcaU**T GTT GTA-3′)·(3′-GTG TAA T**dcaU**A CAA CAT-5′), was significantly enhanced when one equivalent of Gd^{III} ions was added (ΔT_m = +16.1 °C). Titration of Gd^{III} ions and control experiments suggested that the duplex stabilization results from the quantitative formation of a single **dcaU**–Gd^{III}–**dcaU** base pair. In contrast to the case with $\mathbf{U^{OH}}$ and **caU** base, only a single **dcaU**–Gd^{III}–**dcaU** base pairing stabilized the DNA duplex. CD spectroscopic analysis showed that the **dcaU**–Gd^{III}–**dcaU** base pair was accommodated within the duplex without severe structural distortion, although the Gd^{III} complex is likely to adopt a nonplanar bulky conformation. Since **dcaU** functions as a tetradentate ligand, Gd^{III} with high coordination number is appropriate for metal-mediated base pairing with **dcaU**.

The **dcaU** base also forms a hydrogen-bonded **dcaU**–A base pair with a natural A base. In a manner similar to $\mathbf{U^{OH}}$–A and **caU**–A base pairs, the **dcaU**–A base pair was destabilized by adding Gd^{III} ions. The thermal stability of a duplex (5′-CAC ATT A**dcaU**T GTT GTA-3′)·(3′- GTG TAA TAA CAA CAT-5′), containing a **dcaU**−A pair, was decreased (T_m = −3.5 °C) in the presence of equimolar Gd^{III} ions. As summarized in Fig. 8g, the duplex containing a **dcaU**–A pair (T_m = 38.0 °C) is more stable than the duplex with a **dcaU**–**dcaU** mismatch pair (22.5 °C) in the absence of Gd^{III} ions. When Gd^{III} ions are present, the duplex with a **dcaU**–Gd^{III}–**dcaU** pair (38.6 °C) becomes more stable than the duplex with a **dcaU**–A (34.5 °C). The metal-mediated changes in stability of the two duplexes are accompanied by the switching between the hydrogen-bonded and the metal-mediated base pairs. This characteristic property of the bifacial nucleobases would be applicable to the construction of a wide range of metal-responsive DNA supramolecules.

Development of metal-responsive DNA supramolecules with bifacial nucleobases

The metal-responsive bifacial nucleobases, **U^{OH}**, **caU**, and **dcaU**, form both hydrogen-bonded and metal-mediated base pairs in DNA duplexes. Since the base-pairing partner of the bifacial bases can be switched depending on the coexisting metal ions, these bifacial bases can be utilized to endow DNA structures with metal-responsiveness. We first applied the bifacial **U^{OH}** bases to induce DNA strand displacement reactions (SDRs) by the addition of Gd^{III} ions [80]. SDRs are reactions in which one of the strands in a pre-hybridized duplex (termed as an incumbent strand) is replaced with an input single-stranded DNA (termed as an invader strand) [81, 82]. Typical SDRs start at a single-stranded segment called a toehold where the invading strand binds. It is safe to say that SDR is one of the most fundamental processes in the operation of DNA-based molecular machines and molecular computing circuits. The development of SDRs triggered by external stimuli other than DNA/RNA molecules has been considered as an important step toward practical applications of DNA molecular systems in various chemical environments.

We investigated metal-triggered SDRs based on Gd^{III}-mediated switching between **U^{OH}**–A and **U^{OH}**–Gd^{III}–**U^{OH}** base pairs (Fig. 9a). To this end, a DNA strand containing four consecutive **U^{OH}** bases at the 5′ end (**1**) was used as an invader strand. At the biggening, a DNA strand with the same base sequence as **1** except for four A bases at the 5′ end (**2**) was hybridized to a complementary strand containing **U^{OH}** bases at the 3′ end (**3**) through **U^{OH}**–A base pairing. The strand **3** and the invader strand **1** were labeled with a fluorophore and a quencher, respectively. Thus, the progression of the SDR can be monitored by fluorescence quenching (Fig. 9b). The fluorescence intensity decreased immediately after the addition of 4 equiv of Gd^{III} ions, showing that the incumbent strand **2** was displaced with the invader strand **1**. The fluorescence intensity was hardly changed when DNA strands containing T bases in place of the bifacial **U^{OH}** bases were utilized. It was revealed that the SDR was triggered by the Gd^{III}-mediated destabilization of the **U^{OH}**–A pairs and the formation of the **U^{OH}**–Gd^{III}–**U^{OH}** base pairs at the terminal of the strands. In addition, the reverse reaction was found to proceed by removing Gd^{III} ions with an equimolar amount of chelating reagent EDTA. Although the Gd^{III}-triggered SDR is slower than

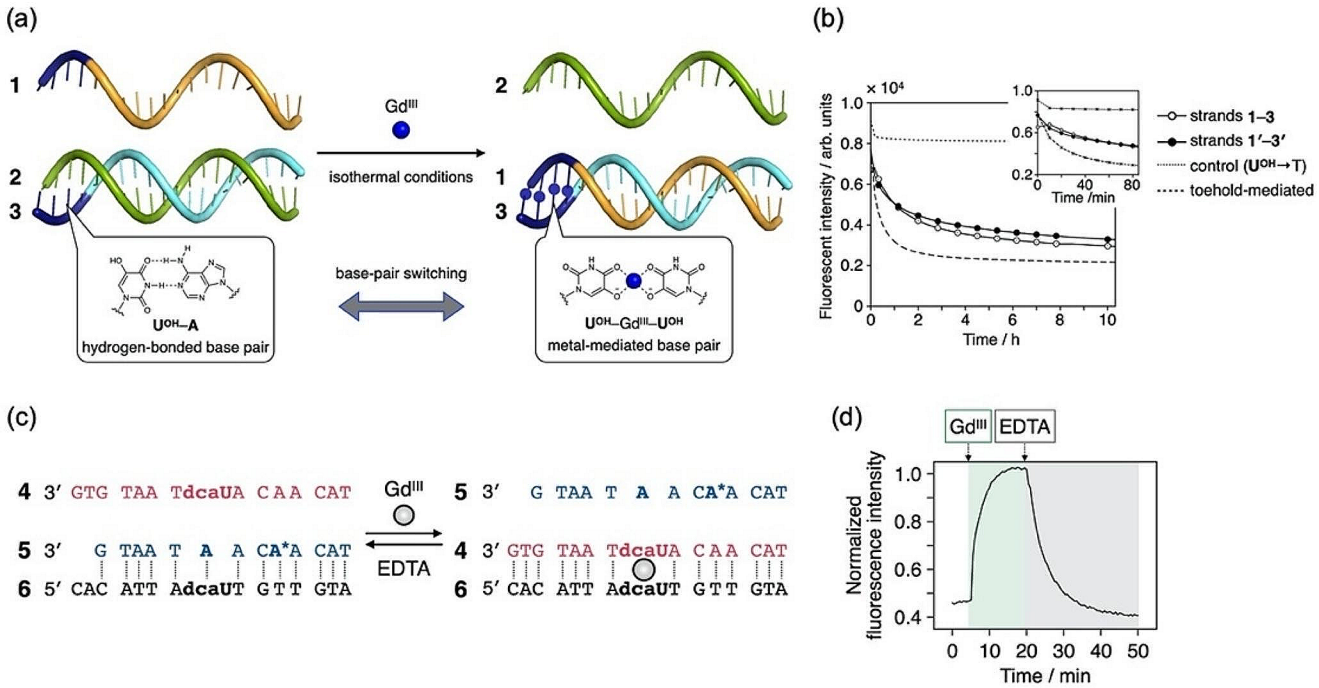

Fig. 9 **a** Schematic representation of metal-mediated DNA strand displacement reactions (SDRs) induced by base-pair switching of **U^{OH}** nucleobases. **U^{OH}** nucleotides in the DNA strands are highlighted. **b** Time-course analysis of Gd^{III}-triggered SDRs. The reaction was started by the addition of Gd^{III} ions (4 equiv). [DNA strand] = 2.0 μM each in 10 mM HEPES buffer (pH 8.0) containing 100 mM NaCl, 25 °C. The strand **3** and the invader strand **1** were labeled with a fluorophore (FAM) and a quencher (Dabcyl), respectively. λ_{ex} = 495 nm, λ_{em} = 519 nm. **c** Gd^{III}-dependent switching of the hybridization partner of **dcaU**-containing strand. "A*" represents a fluorogenic 2-aminopurine nucleobase. **d** Time-course analysis of Gd^{III}-dependent hybridization behaviors of the **dcaU**-containing strand. After annealing the mixture of the three strands, Gd^{III} (1 equiv) and EDTA (5 equiv) were sequentially added. λ_{ex} = 303 nm, λ_{em} = 371 nm, 20 °C. [DNA strand] = 0.5 μM each in 10 mM HEPES buffer (pH 7.0) containing 100 mM NaCl. Panels **a** and **b** are adapted from ref. 80, CC BY 4.0. Panels **c** and **d** are adapted with permission from ref. 79. Copyright 2023 Royal Society of Chemistry

conventional toehold-mediated SDRs, the use of bifacial $\mathbf{U^{OH}}$ bases enabled reversible DNA strand displacement reactions in response to Gd^{III} ions.

Similarly, the hybridization partner of a **dcaU**-containing strand was exchanged by the addition and the removal of Gd^{III} ions (Fig. 9c) [79]. A 15-mer DNA strand containing a **dcaU** base in the middle (**6**) was initially hybridized with its complementary 13-mer strand (**5**) through **dcaU**–A base pairing, and a 15-mer strand with a **dcaU** base (**4**) was used as an invader. Native polyacrylamide gel electrophoresis (PAGE) and fluorescence measurements using the strand **5** containing fluorogenic 2-aminopurine base [83] (Fig. 9d) revealed that the addition of Gd^{III} ions (1 equiv) induced the release of strand **5** and the formation of duplex **4**·**6** containing a **dcaU**–Gd^{III}–**dcaU** pair. Furthermore, the removal of Gd^{III} ions with EDTA resulted in a strand exchange in the reverse direction. The strand exchange was repeated three times by alternate addition and removal of Gd^{III} ions although the efficiency was gradually decreased.

Metal-meditated dissociation of oligonucleotides with the bifacial nucleobases was also applied to the regulation of the DNA triplex formation [84]. The triple-stranded structures are formed by sequence-specific binding of a triplex-forming oligonucleotide (TFO) to a target DNA duplex via Hoogsteen base pairing between a pyrimidine and a purine bases. Since the triplex formation is applicable to gene regulation and dynamic control of DNA structures [85], the development of stimuli-responsive TFOs has been of great interest. We designed TFOs containing $\mathbf{U^{OH}}$ bases to reversibly regulate the triplex formation in a Gd^{III}-dependent manner (Fig. 10). The $\mathbf{U^{OH}}$-modified TFOs such as 5′-TC^{m}C^{m} TT$\mathbf{U^{OH}}$ TC^{m}T $\mathbf{U^{OH}}$TC^{m} T$\mathbf{U^{OH}}$T TTC^{m} C^{m}TT-3′ (C^{m} = 5-methylcytosine) were found to bind to target duplexes by forming $\mathbf{U^{OH}}$·A–T base triads, similar to the natural T·A–T base triads. Melting analysis revealed that the binding affinity of the $\mathbf{U^{OH}}$-modified TFO decreases upon addition of Gd^{III} ions. This is probably because the Hoogsteen $\mathbf{U^{OH}}$·A base pairs were disrupted by the site-selective binding of Gd^{III} to the $\mathbf{U^{OH}}$ bases. The Gd^{III}-responsive triplex formation was then studied by native PAGE. Under the Gd^{III}-free conditions, the mixture of the target duplex and the $\mathbf{U^{OH}}$-containing TFO gave the triplex structure in 73% yield. After the incubation with Gd^{III} ions (6 equiv), the amount of the triplex was dropped to 25% in 2 h, suggesting the release of the TFOs. Subsequent addition of EDTA (6 equiv), which removes Gd^{III} ions, resulted in re-association of the TFO to form the triplex. The binding of the TFOs was cycled at least twice albeit with gradually decreasing the efficiency.

We have recently utilized $\mathbf{U^{OH}}$ bases to construct one of the simplest prototypes of DNA molecular machines [80], so-called DNA tweezers, which take closed and open forms depending on the external stimuli [86]. The $\mathbf{U^{OH}}$-modified DNA tweezers were designed to open and close in response to Gd^{III} ions (Fig. 11a) [80]. The tweezer structure consists of three DNA strands forming two arms (**a**, **b**, and **c**) and

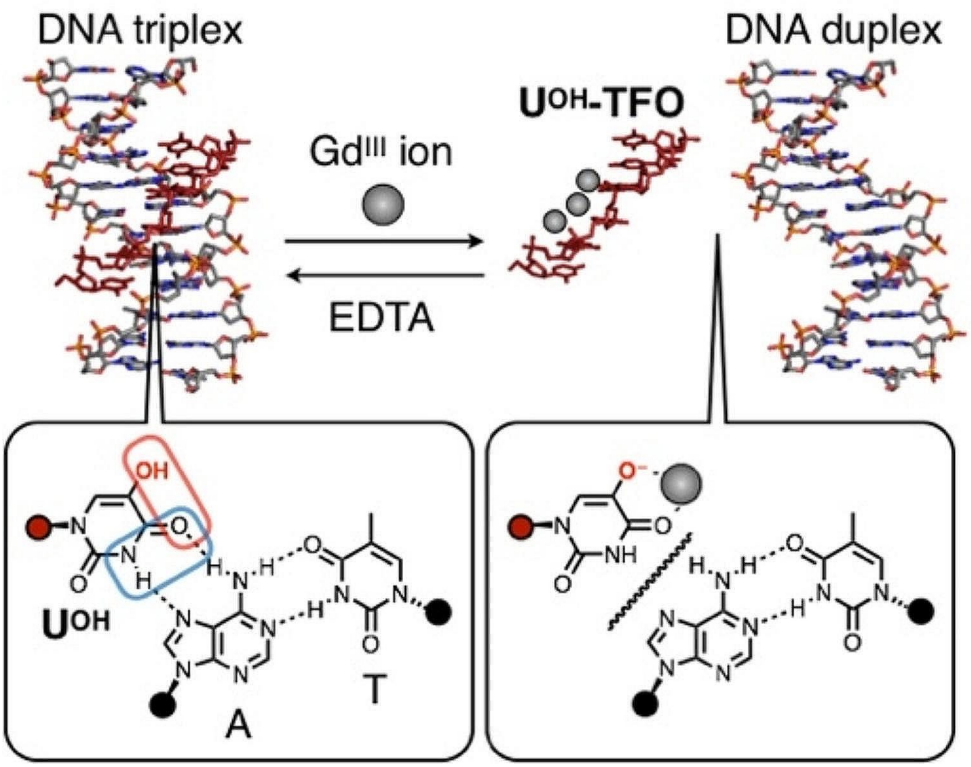

Fig. 10 Schematic representation of Gd^{III}-responsive reversible binding of triplex-forming oligonucleotides (TFOs) with 5-hydroxyuracil ($\mathbf{U^{OH}}$) nucleobases. The chemical structure of a $\mathbf{U^{OH}}$·A–T base triad is shown. $\mathbf{U^{OH}}$·A: Hoogsteen base pair, A–T: Watson–Crick base pair. Reprinted with permission from ref. 84. Copyright 2021 Royal Society of Chemistry

a strand that functions as a stopper (**d**). Four $\mathbf{U^{OH}}$ bases were incorporated at both termini of the stopper strand **d** to close the tweezers via hydrogen-bonded $\mathbf{U^{OH}}$–A base pairing. The addition of Gd^{III} was expected to induce switching from $\mathbf{U^{OH}}$–A to $\mathbf{U^{OH}}$–Gd^{III}–$\mathbf{U^{OH}}$ base pairs, resulting in the release of strand **d** and the opening of the DNA tweezers. Native PAGE analysis confirmed that the closed structure with the stopper **d** was predominantly formed in the absence of Gd^{III} ions while the open state was formed by the addition of Gd^{III} ions. The yield of the open form increased as the amount of Gd^{III} ions increased, reaching ca. 90% with 12 equiv of Gd^{III}. FRET analysis with the hinge strand **b** labeled with a fluorophore and a quencher at the both termini demonstrated Gd^{III}-mediated operation of the DNA tweezers under isothermal conditions (Fig. 11b). The fluorescence intensity increased immediately after the addition of Gd^{III} ions (12 equiv), showing the Gd^{III}-triggered opening of the tweezers. Subsequent addition of EGTA (12 equiv), a chelating agent to remove Gd^{III} ions, led to the decrease in the fluorescence, confirming that the DNA tweezers were closed again. Sequential addition and removal of Gd^{III} ions successfully repeated the opening and closing of the DNA molecular tweezers. Although Gd^{III}–EGTA complexes accumulated in the solution, the metal-mediated base-pair switching between $\mathbf{U^{OH}}$–A and $\mathbf{U^{OH}}$–Gd^{III}–$\mathbf{U^{OH}}$ was proven to be versatile way to operate DNA-based molecular machines.

The bifacial nucleobases can be also utilized to develop metal-responsive allosteric DNAzymes. A Gd^{III}-responsive DNAzyme was logically designed by applying the switching between $\mathbf{U^{OH}}$–A and $\mathbf{U^{OH}}$–Gd^{III}–$\mathbf{U^{OH}}$ base pairs (Fig. 11c) [80]. Three consecutive $\mathbf{U^{OH}}$–$\mathbf{U^{OH}}$ mismatches were introduced into the stem region of a known RNA-cleaving NaA43 DNAzyme [62]. The surrounding bases were redesigned to form a catalytically inert structure in the absence of Gd^{III} ions through $\mathbf{U^{OH}}$–A base pairing. The $\mathbf{U^{OH}}$-modified DNAzyme ($\mathbf{U^{OH}}$**-Dz1**) was expected to undergo an intramolecular structural change upon Gd^{III} addition, from the inactive state containing $\mathbf{U^{OH}}$–A base pairs to the active state containing $\mathbf{U^{OH}}$–Gd^{III}–$\mathbf{U^{OH}}$ pairs. The detailed base sequence was rationally designed with the help of the NUPACK software [87], which predicts possible secondary structures from the base sequences. In the simulation, the $\mathbf{U^{OH}}$–A base pairs in the inactive form were replaced with natural T–A base pairs, and the potential $\mathbf{U^{OH}}$–Gd^{III}–$\mathbf{U^{OH}}$ base pairs in the active form were replaced with G–C base pairs. The RNA-cleaving activity of $\mathbf{U^{OH}}$**-Dz1** was enhanced 14.4-fold by the addition of 3 equiv of Gd^{III} ions (Fig. 11d) while that of the unmodified NaA43 DNAzyme was increased only 1.9-fold. A control DNAzyme in which all $\mathbf{U^{OH}}$ bases were replaced with thymine (T) bases did not cleave the RNA substrate.

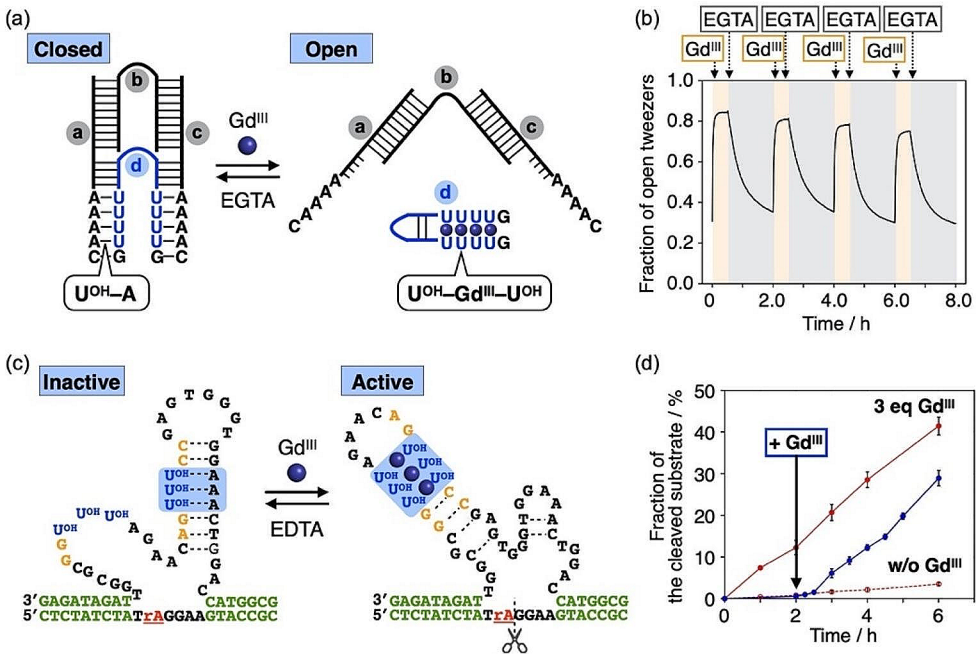

Fig. 11 **a** Schematic representation of Gd^{III}-responsive DNA tweezers. **U** represents $\mathbf{U^{OH}}$ nucleotides. **b** Repeated opening and closing of the $\mathbf{U^{OH}}$-modified DNA tweezers observed by fluorescence analysis. Strand **b** was labeled with a fluorophore (FAM) and a quencher (Dabcyl) at both termini. $\lambda_{ex} = 495$ nm, $\lambda_{em} = 519$ nm, 25 °C. [DNA] = 2.0 μM each in 10 mM HEPES buffer (pH 8.0) containing 100 mM NaCl and 3 mM $MgCl_2$, [$GdCl_3$] = [EGTA] = 24 μM. **c** Molecular design of the $\mathbf{U^{OH}}$-modified DNAzyme ($\mathbf{U^{OH}}$**-Dz1**). "rA" in the substrate represents an adenosine ribonucleotide at the cleavage site. Both the catalytically inactive form (without Gd^{III}) and the active form (with Gd^{III}) are shown. **d** Activation of $\mathbf{U^{OH}}$**-Dz1** by the addition of Gd^{III} ions (3 equiv). The activity of $\mathbf{U^{OH}}$**-Dz1** in the presence and the absence of Gd^{III} ions is also shown. $N = 3$. Error bars indicate the standard errors. Adapted from ref. 80, CC BY 4.0

UV spectroscopic analysis of **U^{OH}-Dz1** confirmed the metal complexation of the **U^{OH}** bases. These results suggest that the activity of **U^{OH}-Dz1** was allosterically enhanced by the formation of **U^{OH}**–Gd^{III}–**U^{OH}** base pairs. Furthermore, the DNAzyme activity was reversibly regulated by adding Gd^{III} or EDTA during the reactions. It was revealed that **U^{OH}-Dz1** showed more efficient switching capability (k_{Gd+}/k_{Gd-} = 14.4) than the Cu^{II}-responsive cognate DNAzyme **H-Dz4** (k_{Cu+}/k_{Cu-} = 5.9). This is owing to the unique design strategy based on the bifacial nature of the **U^{OH}** bases, whereby both the inactive and the active states are stably formed through the formation of **U^{OH}**–A and **U^{OH}**–Gd^{III}–**U^{OH}** base pairs, respectively.

As described above, switching between hydrogen-bonded and metal-mediated base pairs allows for metal-dependent control of DNA hybridization as well as structure conversion. Thus, these bifacial bases would be versatile building blocks for developing metal-responsive DNA supramolecules and nanodevices. The DNA sequences can be designed by modifying existing functional DNAs, more specifically, by replacing natural T bases with **U^{OH}**, **caU**, or **dcaU**. The unique base-pairing properties of the bifacial nucleobases stem from the metal coordination site on the opposite side of the Watson–Crick face. Therefore, bifacial nucleobases with a different metal affinity could be developed by introducing other coordination functionalities at the 5-position of uracil (U) or cytosine (C) bases. We believe that the concept of the metal-responsive bifacial nucleobases would offer new strtegies in the field of DNA supramolecular chemistry and nanotechnology.

Metal-triggered transformation of DNA three-way junction structures

In order to construct DNA-based functional nanoarchitectures, it is important to control the self-assembly of not only duplexes but also branched structures [88]. A DNA three-way junction (3WJ) is a branching structural motif in which three DNA duplexes emanate from its branching point (Fig. 12a). In a manner similar to DNA duplexes stabilized by metal-mediated base pairing, 3WJ structures can be thermally stabilized by interstrand metal complexation (Fig. 12c) [89, 90]. Toward this end, a nucleoside modified with a metal ligand is incorporated into each strand (Fig. 12d). The ligand-modified 3WJs are stabilized through 3:1 ligand–metal complexation in the presence of specific metal ions.

We synthesized bipyridine (bpy)-modified 3WJ structures and investigated metal-dependent stabilization [91–93]. The reported crystal structures of natural 3WJs [94, 95] showed that the 2′-hydrogen of the deoxyribose moiety points to the center of the 3WJ (Fig. 12b). Therefore, the metal ligand was attached to the 2′-position to form the 3:1 metal complex at the junction core. We designed a series of bpy-modified nucleosides, **U_{bpy}-1** [91], **U_{bpy}-2** [92], and **U_{bpy}-3** [93], with different linker moieties. The DNA strands containing a **U_{bpy}-1** nucleotide were synthesized by post-synthetic azide–alkyne Huisgen cycloaddition between oligonucleotides containing a 2′-propargyl uridine and an azide-modified bpy ligand [91]. A DNA 3WJ structure with three bpy ligands at the branching point was constructed by hybridizing three bpy-modified strands (Fig. 12e). Melting experiments showed that the thermal stability of the bpy-modified 3WJs was increased by the addition of an equimolar amount of Ni^{II} ions (ΔT_m = + 8.9 °C) (Fig. 12f). The Ni^{II}-dependent stabilization of the 3WJ was ascribed to the formation of an interstrand tris-bipyridine complex, $Ni^{II}(bpy)_3$, which was confirmed by UV spectroscopic analysis and control experiments. The addition of transition metal ions other than Ni^{II} was found to stabilize the bpy-modified 3WJ as well (ΔT_m = + 5.0 °C for Fe^{II}; +3.3 °C for Co^{II}). It is interesting to note that the degree of the metal-dependent stabilization ($Ni^{II} > Fe^{II} > Co^{II}$) followed the order of the overall stability constants of $M^{II}(bpy)_3$ complexes (log β_3 = 20.2, 17.2, and 15.9 for Ni^{II}, Fe^{II}, and Co^{II}, respectively [96]). Afterward, Wagenknecht et al. reported a 3WJ structure with an analogous bpy-modified cytidines, which was also stabilized by the formation of $Ni^{II}(bpy)_3$ [97].

Larger stabilization was observed for 3WJ structures containing the improved version of bpy-modified nucleosides, **U_{bpy}-2** [92] and **U_{bpy}-3** [93]. We hypothesized that the triazole group of **U_{bpy}-1** could interfere with the metal coordination process and the relatively rigid linker may disturb the local structure of the junction core, thus adversely affecting the 3WJ stability. Accordingly, **U_{bpy}-2** and **U_{bpy}-3** were designed to have the bpy ligand with a carbamate or an amide linker, respectively. **U_{bpy}-2** and **U_{bpy}-3** have opposite stereochemistry of the modification sites (i.e., the 2′-position), but the linkers have the same length. The thermal stability of the 3WJ containing **U_{bpy}-2** was significantly enhanced upon addition of one equivalent of Ni^{II} ions (ΔT_m = + 18.8 °C) (Fig. 12f). Likewise, the 3WJ containing **U_{bpy}-3** was greatly stabilized by adding Ni^{II} ions (ΔT_m = + 17.7 °C). These results demonstrated that the stereochemistry of the modification site has little influence on the thermal stability of the bpy-modified 3WJs.

Recently, DNA 3WJ structures modified with phenanthroline (phen) ligands were synthesized by using a newly designed phen-modified nucleosides **U_{phen}** [98]. The 3WJ containing three phen ligands at the center was found to be stabilized through the formation of an interstrand $Ni^{II}(phen)_3$ complex (ΔT_m = + 16.9 °C) (Fig. 12f). The phen ligands are known to form 3:1 metal complexes with higher stability constants than bpy (e.g., log β_3 = 24.3 for $Ni^{II}(phen)_3$ [96]).

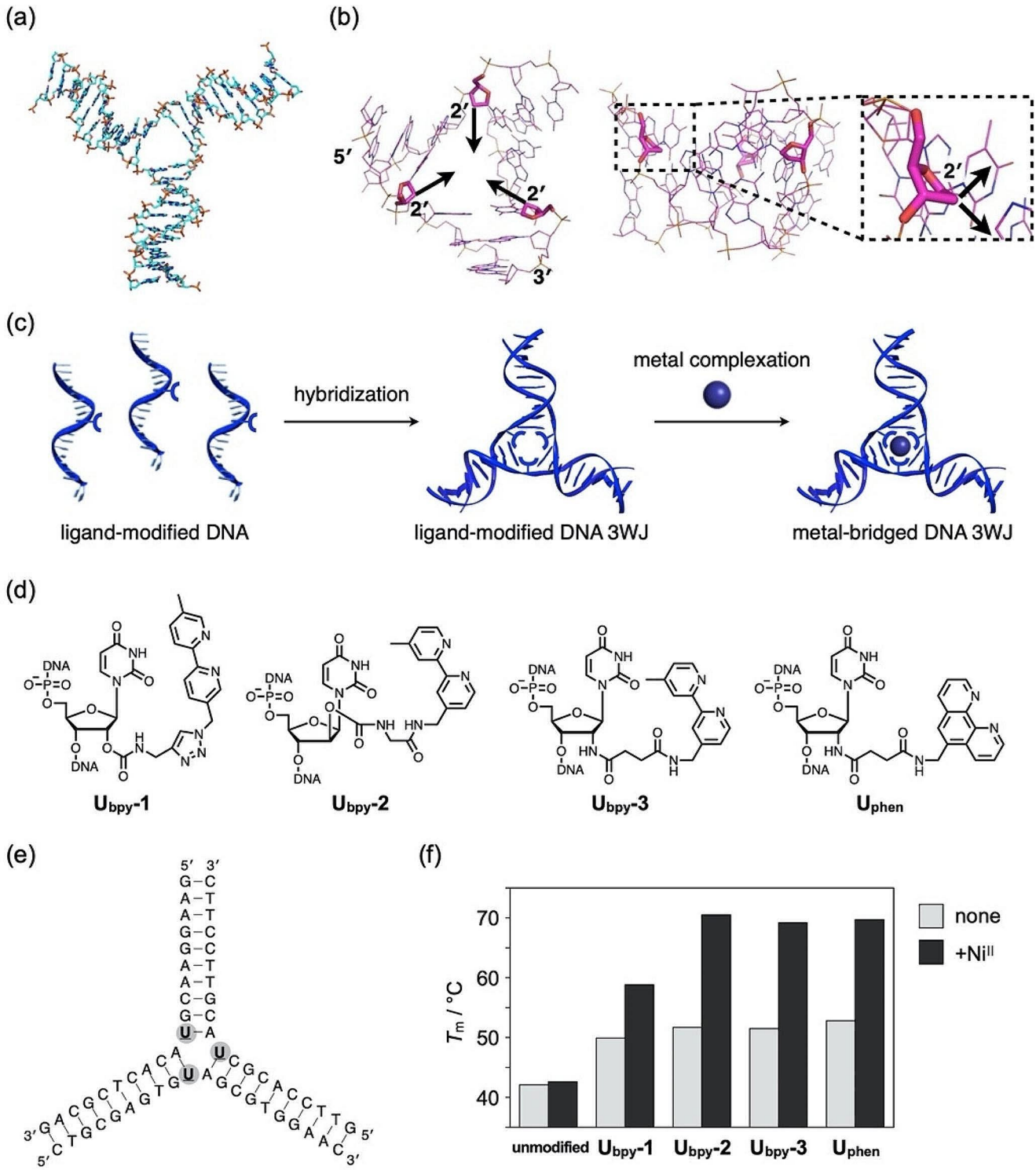

Fig. 12 **a** An ideal structure of an unmodified DNA three-way junction (3WJ). Dipicted based on a crystal structure reported by Baldwin et al. (PDB ID: 1DRG) [95]. **b** Molecular structure of an unmodified DNA 3WJ motif. Drawn based on a crystal structure reported by Hannon et al. (PDB ID: 2ET0) [94]. **c** Schematic representation of metal-dependent stabilization of ligand-modified DNA 3WJ structures. **d** Molecular design of ligand-modified nucleotides. **e** Base sequences of the ligand-modified DNA strands forming 3WJs. **U** represents the ligand-modified nucleotides, $\mathbf{U_{bpy}}$**-1**, $\mathbf{U_{bpy}}$**-2**, $\mathbf{U_{bpy}}$**-3**, or $\mathbf{U_{phen}}$. **f** Melting temperatures (T_m) of the unmodified 3WJ and the ligand-modified 3WJs in the absence and the presence of Ni^{II} ions. $[Ni^{II}]/[3WJ] = 1.0$ in 10 mM MOPS buffer (pH 7.0) containing 100 mM NaCl. Panel **b** is reproduced with permission from ref. 93. Copyright 2021 Wiley-VCH

Nonetheless, the degree of the Ni^{II}-dependent stabilization of the phen-modified 3WJ was comparable to that of the bpy-modified 3WJs. The $\mathbf{U_{phen}}$ nucleoside has the same succinate linker as $\mathbf{U_{bpy}}$**-3**, but the positions of the coordinating nitrogen atoms are different. This slight structural difference might affect the degree of the metal-mediated 3WJ stabilization.

The Ni^{II}-mediated stabilization of the ligand-modified DNA 3WJs was further applied to metal-triggered structural transformation between duplexes and 3WJs (Fig. 13) [92, 93, 96]. For example, a mixture of three DNA strands containing a $\mathbf{U_{bpy}}$**-2** nucleoside (**L1**, **L2**, and **L3**) that can form the bpy-modified 3WJ and their complementary natural strands (**S4**, **S5**, and **S6**) were annealed in the absence and the presence of Ni^{II} ions [92]. Native polyacrylamide gel electrophoresis (PAGE) analysis showed that the six DNA strands self-assembled into three DNA duplexes (**L1S4**, **L2S5**, and **L3S6**) under Ni^{II}-free conditions. When one equivalent of Ni^{II} ions was added, the formation of two kinds of 3WJ structures, namely, a metal-bridged 3WJ (**L1L2L3**·Ni^{II}) and an unmodified 3WJ (**S4S5S6**) was observed. The yields of the 3WJs were estimated to be about 60%. These results demonstrated that structure conversion from duplexes to 3WJs occurred via the formation of an interstrand $Ni^{II}(bpy)_3$ complex. More efficient duplex-to-3WJ conversion was realized by redesigning the base sequences of the counter natural strands (**M4**, **M5**, and **M6**) (Fig. 13a). Two nucleobases at the junction core were altered to introduce mismatches, which decreased the stability of the duplexes. As a result, the efficiency of the Ni^{II}-mediated 3WJ formation was improved to ca. 90% (Fig. 13b). Furthermore, the addition of EDTA to remove Ni^{II} ions resulted in the regeneration of the duplexes, showing reversible metal-responsive conversion between the duplexes and the 3WJs.

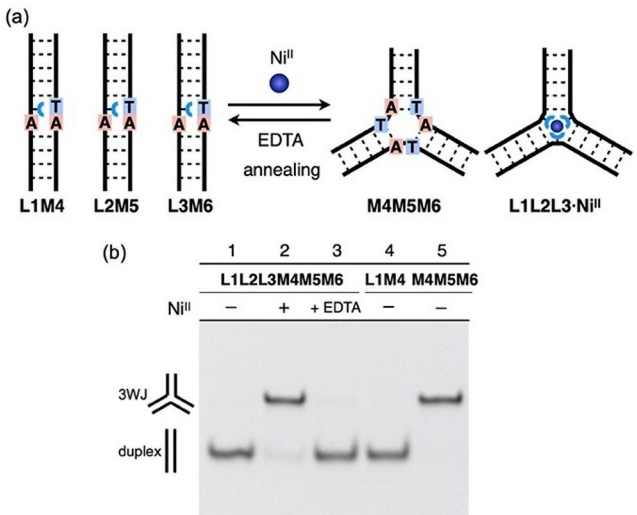

Fig. 13 **a** Schematic representation of Ni^{II}-mediated structural transformation between DNA duplexes and 3WJs. **L1**, **L2**, and **L3** contain a $\mathbf{U_{bpy}}$**-2** nucleotide in the middle. **M4**, **M5**, and **M6** have the complementary base sequences except for two bases in the middle. **b** Native polyacrylamide gel electrophoresis (PAGE) of a mixture of **L1**, **L2**, **L3**, **M4**, **M5**, and **M6** in the absence and the presence of Ni^{II} ions (1.0 equiv). **M4** was labeled with a fluorophore (FAM). [DNA strand] = 1.0 μM each in 10 mM MOPS buffer (pH 7.0) containing 100 mM NaCl, [Ni^{II}] = 1.0 μM or [EDTA] = 10 μM. Reproduced with permission from ref. 92. Copyright 2016 Royal Society of Chemistry

Ni^{II}-triggered structure conversion was also demonstrated with 3WJ containing $\mathbf{U_{bpy}}$**-3** [93]. With optimized base sequences, the duplexes were formed exclusively in the absence of Ni^{II} ions, and the 3WJs were formed in 83% yield by the addition of Ni^{II} ions. In the case of the phen-modified DNA strands containing $\mathbf{U_{phen}}$ [98], the addition of Ni^{II} ions did not induce the formation of 3WJs when unmutated complementary strands (i.e., **S4**, **S5**, and **S6**) were used. This is probably due to the high stability of the duplexes, in which the large aromatic moiety of the phen ligand intercalates into the base pairs. When the mutated strands (**M4**, **M5**, and **M6**) were utilized, the 3WJs were formed in 88% yield in the presence of Ni^{II} ions while duplexes were mainly formed without Ni^{II} ions.

The 3WJ stabilization via $Ni^{II}(bpy)_3$ complexation was also applied to metal-mediated self-sorting of 3WJ structures (Fig. 14) [93]. Self-sorting is a process wherein molecules in a complex mixture discriminate self from non-self to selectively self-assemble into specific structures [99]. We have demonstrated Ni^{II}-mediated self-sorting of 3WJs by using DNA strands containing a $\mathbf{U_{bpy}}$**-3** nucleotide in the middle (**L1**, **L2**, and **L3**). Their unmodified counterparts (**S1′**, **S2′**, and **S3′**) were tailed with oligo-thymidine so that the different 3WJs exhibit different mobilities in native PAGE analysis (Fig. 14a). When all the six strands were annealed in the absence of Ni^{II} ions, at least three bands were observed on the gel, indicating that 3WJs with zero to three bpy ligands (**S1′S2′S3′**, **S1′S2′L3**, **S1′L2L3**, etc.) were randomly formed (Fig. 14b). In the presence of one equivalent of Ni^{II} ions, only two bands appeared, confirming the selective formation of the metal-bridged 3WJ **L1L2L3**·Ni^{II} and the unmodified 3WJ **S1′S2′S3′**. Taken together, the self-sorting of the 3WJs was successfully demonstrated by $Ni^{II}(bpy)_3$ complexation at the junction core.

DNA 3WJ structures are one of the fundamental building blocks for synthesizing DNA nanoarchitectures such as DNA polyhedra and three-dimensional networks [88]. DNA hydrogels [100] and nanodroplets [101] can be made based on the 3WJ formation as well. Thus, metal-mediated stabilization and structure induction of ligand-modified 3WJs are expected to offer novel approaches to develop metal-responsive DNA materials. For example, DNA polyhedra [102, 103] containing ligand-modified 3WJs as their vertices would be formed in a metal-dependent manner. It is expected that metal complexation increases the rigidity of

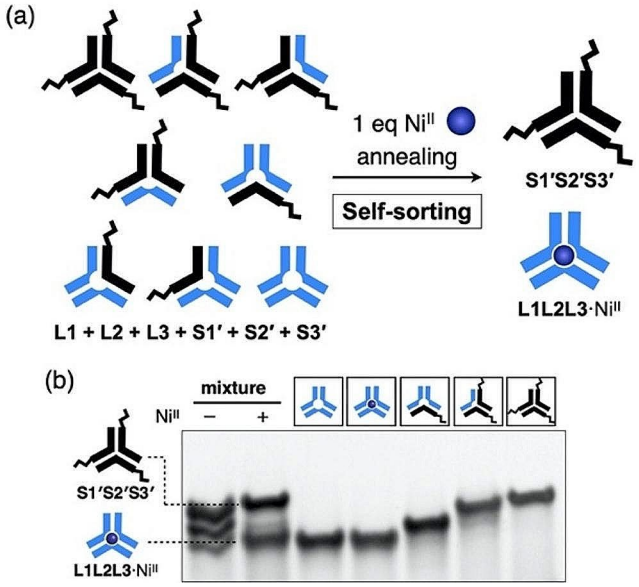

Fig. 14 Ni^{II}-mediated self-sorting of 3WJs. **a** Schematic representation of the self-sorting. Blue: DNA strands containing a $\mathbf{U}_{bpy}$**-3** (**L1**, **L2**, and **L3**), black: unmodified strands with an oligo-T tail (**S1′**, **S2′**, and **S3′**). **b** Native polyacrylamide gel electrophoresis (PAGE) of a mixture of **L1**, **L2**, **L3**, **S1′**, **S2′**, and **S3′** in the absence and the presence of Ni^{II} ions (1.0 equiv) after staining with SYBR Gold. The 3WJs **L1L2L3**, **L1L2 L3**·Ni^{II}, **L1L2S3′**, **L1S2′S3′**, and **S1′S2′S3′** were also analyzed as markers. [DNA strand] = 1.0 μM each in 10 mM MOPS buffer (pH 7.0) containing 100 mM NaCl, [Ni^{II}] = 1.0 μM or [EDTA] = 10 μM. Reproduced with permission from ref. 93. Copyright 2021 Wiley-VCH

the 3WJ structures. Thus, the physical property of DNA hydrogels and droplets composed of ligand-modified 3WJs could be changed in response to metal ions. In theory, metal selectivity can be altered by careful selection of the ligand moiety. Therefore, the metal-responsive DNA 3WJs will be utilized as novel building blocks in future studies of DNA supramolecular chemistry and nanotechnology.

Conclusion

In this review, the recent progress on the development of metal-responsive DNA supramolecules were described. Metal-mediated base pairs, formed through metal complexation of opposing ligand-type nucleobase analogs, have been employed to develop metal-responsive allosteric DNAzymes (catalytic DNA). Split DNAzymes and single-stranded DNAzymes were rationally designed so that metal-mediated base pairing reorganizes the catalytically active structures. In the case of the non-split single-stranded DNAzymes, the base sequences were strategically designed to block the catalytic domain in the absence of the metal ions. As a result, the DNAzyme activity can be reversibly regulated in response to the specific metal ions. Notably, an AND-gate DNAzyme activated only in the presence of both Ag^{I} and Cu^{II} ions was created based on the same strategy. This design strategy would be applicable to embedding metal-responsiveness to various types of functional DNAs and DNA nanoarchitectures.

The novel concept of metal-responsive base-pairing system was proposed by using bifacial nucleobases that form both hydrogen-bonded and metal-mediated base pairs. The bifacial nucleobases such as $\mathbf{U}^{\mathbf{OH}}$, **caU**, and **dcaU** were designed to have a multidentate metal-binding site in addition to the Watson–Crick hydrogen-bonding site. While DNA duplexes are thermally stabilized by the formation of the metal-mediated base pairs (such as $\mathbf{U}^{\mathbf{OH}}$-Gd^{III}–$\mathbf{U}^{\mathbf{OH}}$), duplexes containing hydrogen-bonded base pairs (such as $\mathbf{U}^{\mathbf{OH}}$–A) are destabilized by the addition of the corresponding metal ions. This unique property of the bifacial nucleobases allows for the metal-triggered switching between the hydrogen-bonded and the metal-mediated base pairs. Subsequently, the metal-mediated base-pair switching was applied to metal-triggered DNA strand displacement reactions (SDRs), which would be employed to metal-dependent operation of DNA-based molecular machines and computing systems. As a simplest model, the DNA tweezers that open and close in response to Gd^{III} ions were successfully constructed. The Gd^{III}-responsive allosteric DNAzyme was also developed by the incorporation of $\mathbf{U}^{\mathbf{OH}}$ bases into a known DNAzyme sequence. Since the duplex stability and the hybridization behavior can be drastically changed by metal complexation, the metal-mediated base-pair switching of the bifacial nucleobases would be useful for dynamic control of DNA nanostructures.

Metal-dependent stabilization of DNA structures other than duplexes is also an important topic. Metal-bridged DNA three-way junction (3WJ) structures were constructed by using DNA strands containing a bipyridine-modified or a phenanthroline-modified nucleotide. The metal ligands were introduced at the 2′-position of the nucleoside so that three ligands are preorganized in the center of the junction. The thermal stability of the ligand-modified 3WJs were found to be significantly enhanced through the formation of an interstrand 3:1 metal complex such as Ni^{II}(bpy)$_3$ and Ni^{II}(phen)$_3$. Metal-mediated structural transformation between DNA duplexes and 3WJs was demonstrated by using the ligand-modified DNA strands and their natural complementary strands. Likewise, Ni^{II}-mediated self-sorting of the 3WJ structures was successfully demonstrated. Since DNA branched structures are essential structural motifs to build up DNA nanoarchitectures, the metal-mediated stabilization and structural induction of the metallo-3WJ motifs would have many potential applications in the field of supramolecular nucleic acid chemistry.

Metal-mediated base pairs consisting of natural nucleobases, T–Hg^{II}–T and C–Ag^{I}–C, have been broadly utilized

to develop Hg^{II}- and Ag^{I}-responsive DNA materials [32]. However, the sequence design is often troublesome because Hg^{II} and Ag^{I} ions could bind to T and C bases at unintended positions. In contrast, the incorporation of artificial ligand-bearing nucleotide into DNA structures enables the use of metal ions that rarely interact with natural nucleobases. Our molecular design discussed here would be highly compatible with standard DNA nanotechnologies because only a few modified nucleosides are introduced as additional building blocks. Therefore, we are convinced that the rational design of the metal-responsive DNA systems will largely contribute to a wide range of DNA material sciences, particularly, DNA supramolecular chemistry and DNA nanotechnology. The metal-dependent transformation of DNA nanostructures as well as the metal-mediated operation of DNA molecular machines are now under investigation.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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