# $Cu^{II}$-dependent regulation of a split-DNAzyme having consecutive ethenoadenine nucleobases as metal recognition sites

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Metal-mediated base pairs consisting of opposing ligand-type nucleobases and a bridging metal ion are often applied towards the development of stimuli-responsive DNA architectures. Previously we have developed a $Cu^{II}$-dependent allosteric DNAzyme by incorporating artificial ligand-type nucleobases such as hydroxypyridone (**H**).[1] However, the cumbersome synthesis and low yields associated with artificial nucleosides make the development of metal-responsive DNA systems especially challenging.

In this study, we employed an easily synthesized damaged nucleobase, 1,$N^6$-ethenoadenine (**εA**), as a ligand-type nucleobase for the development of a metal-responsive DNAzyme. It was reported that the formation of a single **εA**–$Cu^{II}$–**εA** base pair stabilizes the DNA duplex ($\Delta T_m$ = +3 °C),[2] but such a low stabilization effect is insufficient for the metal-mediated regulation of DNA structures. We found that incorporation of three consecutive **εA**–$Cu^{II}$–**εA** base pairs into DNA duplexes allows for $Cu^{II}$-dependent significant duplex stabilization ($\Delta T_m$ = +11.5 °C). Motivated by this result, we modified a reported RNA-cleaving DNAzyme (NaA43)[3] by splitting it into two strands and incorporating three **εA**–**εA** mismatch pairs into the stem duplex (Figure). In the absence of $Cu^{II}$ ions, the activity of the **εA**-modified DNAzyme was significantly suppressed. Upon addition of $Cu^{II}$ ions (3 equiv.), the RNA-cleaving activity was enhanced by 5.3-fold. The activity of neither the unmodified NaA43 DNAzyme nor a control DNAzyme having **A**–**A** mismatches was altered by adding $Cu^{II}$ ions. These results indicate that the split **εA**-DNAzyme can hybridize via **εA**–$Cu^{II}$–**εA** base pairing to reconstruct the catalytic core, thereby enhancing the activity in response to $Cu^{II}$ ions.[4]

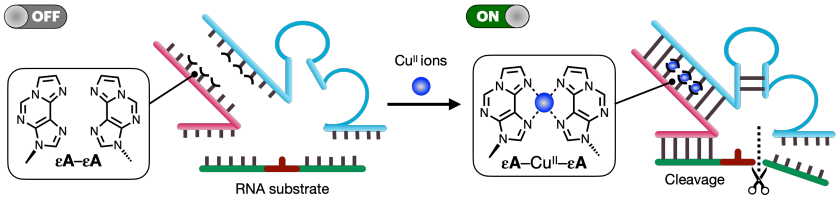


**Figure**. Schematic illustration of a $Cu^{II}$-dependent split DNAzyme regulated by the formation of consecutive **εA**–$Cu^{II}$–**εA** base pairs.

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