Supporting Information

Histidine-DNA Nanoarchitecture as Laccase Mimetic DNAzymes

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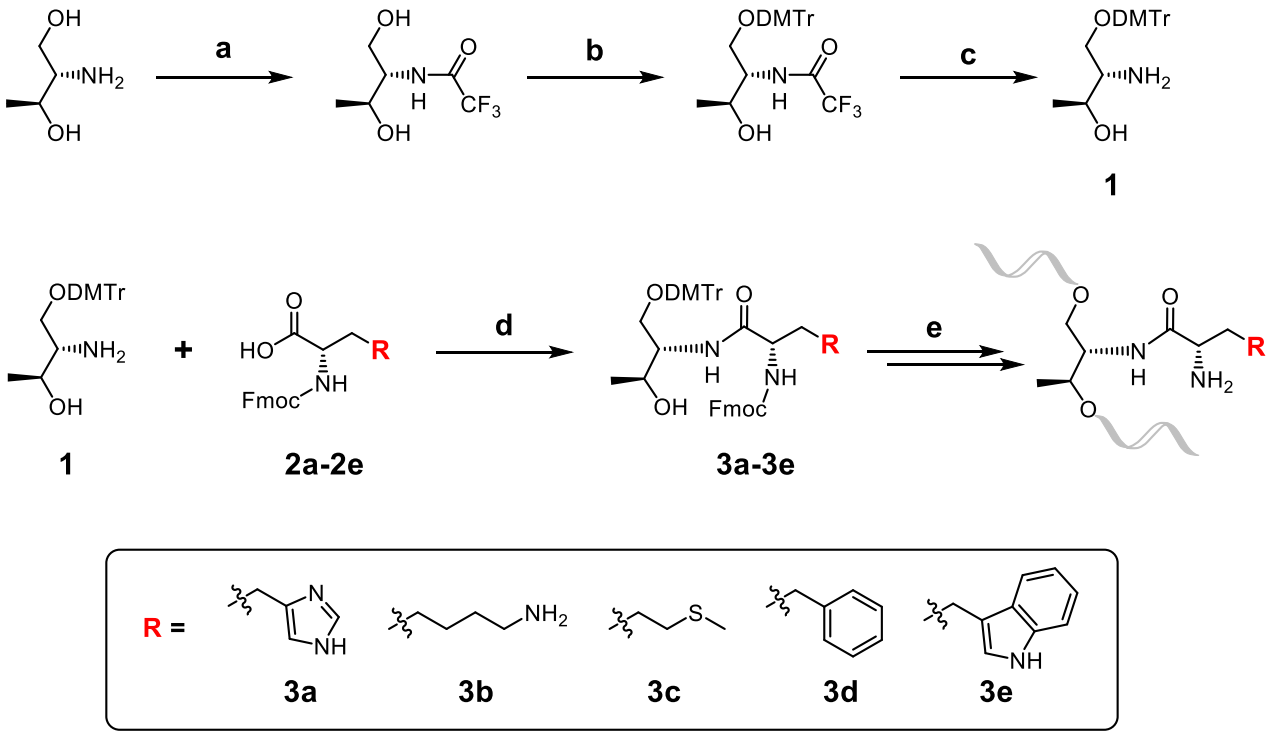
Materials

4,4'-Dimethoxytrityl chloride and Copper nitrate were received from Wako Chemicals and used without further purification. 2-cyanoethyl *N,N*-diisopropylchloro phosphoramidite, D-threoninol (97%), Fmoc-Phe-OH (>97%), Fmoc-His-OH, Fmoc-Lys-OH, Fmoc-Met-OH, Fmoc-Trp-OH were purchased from Sigma-Aldrich Chemicals Co. and used as received. *N,N*-Diisopropylethylamine, *N,N*-Dimethylformamide, trimethylamine, MOPS (pH 7.0) were purchased from Nacalai and used as received. 2,4-dichlorophenol and 4-aminoantipyrine were obtained from TCI. Glen-Pak™ DNA cartridges columns are purchased at Glen research and used. All other chemicals and solvents were purchased from Sigma-Aldrich Chemicals Co., Wako Pure Chemical Ind. Ltd., TCI, or Kanto Chemical Co. Inc. and used without further purification and synthetic oligonucleotides were obtained from Sigma Genosys. Water was deionized (specific resistance of $\geq$ 18.0 MW cm at 25^{o}C) by a Milli-Q system (Millipore Corp.).

Methods and Equipment

NMR spectra were obtained on a JEOL JNM ECA-600 spectrometer operating at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR in $CDCl_3$ unless otherwise noted. Flash column chromatography was performed employing Silica Gel 60 (70–230 mesh, Merck Chemicals). Silica-gel preparative thin-layer chromatography (PTLC) was performed using plates from Silica gel 70 PF_{254} (Wako Pure Chemical Ind. Ltd.). DNA concentrations were measured by NanoDrop ND-1000 spectrophotometer. Purchased 0.5 M MOPS (pH 7.0) were diluted with nuclease free water for the chemical reactions. 2 mM $Cu(NO_3)_2$ solution was prepared by diluting 3.75 mg of $Cu(NO_3)_2$ in 10 mL of mQ and applied to DNA solution with certain equivalents. Cell was observed by confocal microscope (FV3000) from Olympus.

Scheme S1. Synthetic Route of Amino-Nucleic Acids Hybrids (ANHs)[1,2]



Reagents and conditions: (a) $CF_3COOC_2H_5$, dry MeOH, 0 °C, 2 h; (b) DMT-Cl, DIEA, DMAP, CH_2Cl_2, pyridine, 0 °C→r.t. 2.5 h; (c) NH_3, EtOH/H_2O, r.t. 3 days; (d) PyBOP, DIEA, DMF, r.t.,1 day.; (e) $(iPr_2N)_2PO(CH_2)_2CN$, DIEA, CH_3CN, 0 °C→r.t, 1.5 h

*For characterization data including NMR and mass spectroscopy of synthesized ANH modules, please see references S1 and S2.

Oligonucleotide (ODN) Synthesis

ODNs were synthesized on solid supports using ANH building blocks and commercially available $O^{5'}$-dimethoxytrityl -2'-deoxyribonucleoside $O^{3'}$-phosphoramidites. Solid-phase oligonucleotide synthesis was performed on an ABI DNA synthesizer (Applied Biosystem, Foster City, CA) or M-2-MX DNA/RNA synthesizer (Nihon Techno Service Co., Ltd., Tsukuba, Japan). The modified phosphoramidite was chemically synthesized as described above and without purification incorporated into oligonucleotide through coupling reaction for 10 minutes. The coupling yields of ANH phosphoramidites were around 10 % less than the ones obtained with standard phosphoramidite building blocks. Cleavage from the solid support

and deprotection were accomplished with 50:50 of $MeNH_2$ in 40 wt. % in water and NH_3 in 28 wt. % in water at RT for 15 min and then at 65 °C for 15 min. The synthesized oligonucleotides were eluted from Glen-Pak™ DNA purification cartridges with purification steps are performed as per procedure. The final elution was subjected to normal-phase HPLC purification (2 % to 70 % ACN in 50 mM TEAA (pH 7.0) buffer, flow rate of 3.0 mL/min). After purification by HPLC, the products were confirmed by MALDI-TOF MS using a Bruker microflex-KSII (Bruker Corporation, Billerica, MA) (**Table S1**). DNA concentrations were determined by using NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE).

For HPLC analysis, COSMOSIL 5C18 AR-II (Nacalai Tesque, Inc., Kyoto, 150 × 10 mm id), a linear gradient of 2 % to 30 % acetonitrile (in 50 mM TEAA (pH 7.0) buffer) over 30 min at a flow rate of 3.0 mL/min and detection at 254 nm were used.

Table S1. MALDI-TOF-Mass data of ANH-containing ODNs

Name	DNA oligomers	Calcd.	Found
Duplex-H	5'-GGACC-**His**-GGTCC-3'	3333.20	3326.69
Duplex-HH	5'-GGACC-**His**-**His**-GGTCC-3'	3637.50	3631.61
interG4-HH	5'-GG-**His**-**His**-GG-3'	1863.35	1861.87
interG4-FF	5'-GG-**Phe**-**Phe**-GG-3'	1883.43	1875.90
interG4-MM	5'-GG-**Met**-**Met**-GG-3'	1851.44	1848.44
interG4-KK	5'-GG-**Lys**-**Lys**-GG-3'	1845.42	1844.53
interG4-WW	5'-GG-**Trp**-**Trp**-GG-3'	1961.48	1959.36
QD-HH-A	5'-GG-**His**-**His**-GG CGCGAAG-3'	4055.77	4053.96
QD-HH-B	5'-CTTCGCG GG-**His**-**His**-GG-3'	3997.72	3994.81
3WJ-HH-A	5'-GACTAGCC-**His**-**His**-AGTCGCCG-3'	5475.70	5475.34
3WJ-HH-B	5'-CGGCGACT-**His**-**His**-CCGCTTCT-3'	5417.64	5416.55
3WJ-HH-C	5'-AGAAGCGG-**His**-**His**-GGCTAGTC-3'	5579.77	5579.44

Determination of kinetic parameters

The enzymatic parameters of various DNAzymes or laccase were studied by measuring the initial reaction velocity at different 2,4-DP concentrations (1.0, 0.5, 0.25, 0.125, 0.0625, 0.0313, and 0.0156 mM) with 1.0 mM 4-AP. The initial reaction velocity V_0 was determined from the absorption changes of the products according to Eq. 1. The kinetic parameters (V_{max} and K_m) ware calculated by Michaelis-Menten equation (Eq. 2) and Lineweaver-Burk equation (Eq. 3)

$$V_0[mM/sec] = \frac{\Delta A}{\varepsilon} \qquad (1)$$

$$V_0 = \frac{V_{max}[S]}{K_m + [S]} \qquad (2)$$

$$\frac{1}{V_0} = \frac{K_m}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}} \qquad (3)$$

Where ΔA is the change of absorbance at 510 nm, molar absorption coefficient ε is 13.6 $mM^{-1}\cdot cm^{-1}$, V_{max} is the maximum reaction rate, K_m is the Michaelis-Menten constant, and [S] is the concentration of substrates (2,4-DP). We also calculated k_{cat}, defined by Eq. 4, to compare the performance of various DNAzymes and laccase.

$$k_{cat} = \frac{V_{max}}{[E]_0} \qquad (4)$$

Where k_{cat} is the rate constant for conversion of substrate to product and $[E]_0$ is the concentration of various DNAzymes or laccase.

Table S2. Kinetic parameters of various DNAzymes and laccase

Catalyst	V_{max} ($\times 10^{-5}$ mM·min^{-1})	K_m (mM)	k_{cat} (min^{-1})	k_{cat}/K_m (mM^{-1}·min^{-1})
Laccase [a]	1.26	0.11	0.01	0.11
Cu(II) [a]	28.57	0.19	0.03	0.16
duplex-HH [a]	6.79	0.11	0.07	0.60
interG4-HH [b]	3.90	0.15	0.04	0.56
QD-HH [b]	4.31	0.08	0.04	0.56
3WJ-HH [a]	2.24	0.06	0.02	0.39
duplex-TT [a]	11.08	0.09	0.01	0.13

[a] Solution conditions: 20 mM MOPS buffer (pH 7.0), 100 mM NaCl, 0.1 mol% of catalyst.

[b] Solution conditions: 20 mM MOPS buffer (pH 7.0), 100 mM KCl, 0.1 mol% of catalyst.

CD Spectroscopy

CD spectra of oligonucleotide solutions collected in 1 nm steps from 350 nm to 220 nm were measured using JASCO J-805LST Spectrometer in a 1 cm quartz cuvette. Each spectrum shown is the average of two individual scans. The samples with ANH-modified DNA and copper ions were denatured at 95 °C for 5 min and annealed slowly to RT then stored at 25 °C until experiments were initiated. All samples were prepared in a total volume of 120 μL containing 4.0 μM oligonucleotide and 20 mM MOPS buffer (pH 7.0).

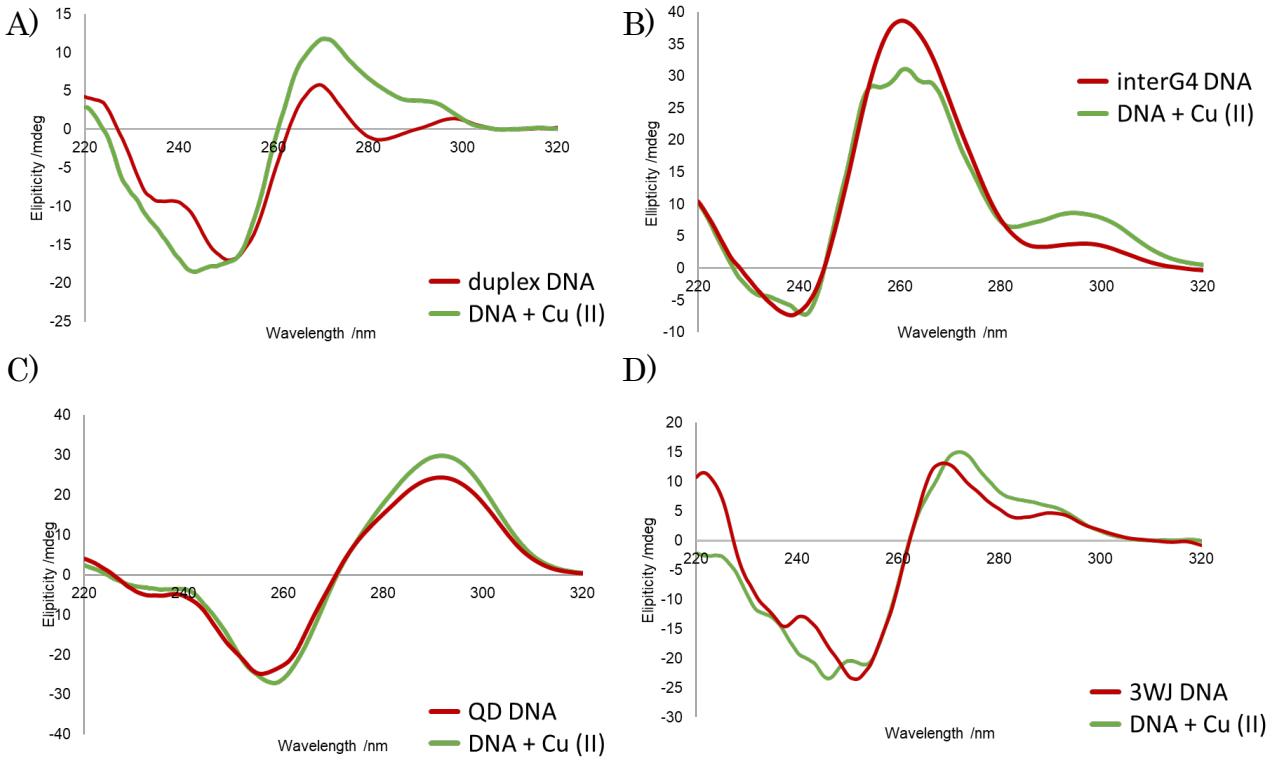


Figure S1. CD spectra of various **His**-modified DNA structures under catalytic conditions. A) duplex, B) intermolecular G4, C) quadruplex-duplex hybrid, D) three-way junction

UV-Melting

Melting temperatures were determined by measuring changes in absorbance at 260 nm for duplex and three-way junction DNAs and 295 nm for interG4 and quadruplex-duplex hybrid DNAs as a function of temperature on a JASCO V-750 spectrophotometer equipped with a JASCO PAC-743R thermocontrolled cell changer and a JASCO CTU-100 thermocirculator. Absorbance was recorded from 15 to 90 °C at a rate of 1 °C/min. The melting samples with ANH-modified DNA and copper ions were denatured at 95 °C for 5 min and annealed slowly to room temperature then stored at 4 °C until experiments were initiated. All melting samples were prepared in a total volume of 120 µL containing 4.0 µM oligonucleotide and 20 mM MOPS buffer (pH 7.0).

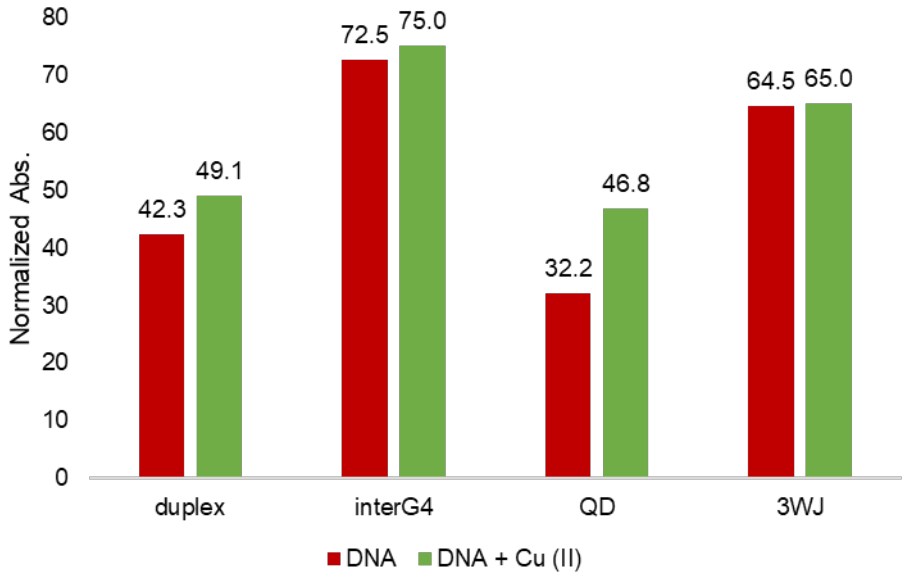


Figure S2. T_m value of various **His**-modified DNA structures under catalytic conditions

UV-Visible Absorbance

Before reaction condition optimization, the product was confirmed by measuring reaction mixture (0.1 mol% DNA + 1.0 mol% $Cu(NO_3)_2$) with JASCO V-750 spectrophotometer from 350 nm to 650 nm at 25 °C, 1 hr (Figure S3). Also, product titration graph (Figure S3C) has been plotted by measuring various concentration of the chromophore, which was synthesized and purified after preparative scale reaction, at 510 nm. The chemical reaction progress (0-12 hr) was measured by UV-vis absorbance spectra at fixed wavelength (510 nm), 25 °C. First, annealed DNAzyme with various equivalent of the copper ion were transferred on 8-well cell of spectrophotometer. Then, 4-AP (10 mM stock solution, 20 μL) and 2,4-DP (10 mM stock solution, 20 μL) solutions were added to DNA solution and gently pipette mixed (final volume of solution is 200 μL). The absorption increase was detected up to 12 hr (or 6 hr) for each 1 minute. All reactions were triplicated and calculated mean values.

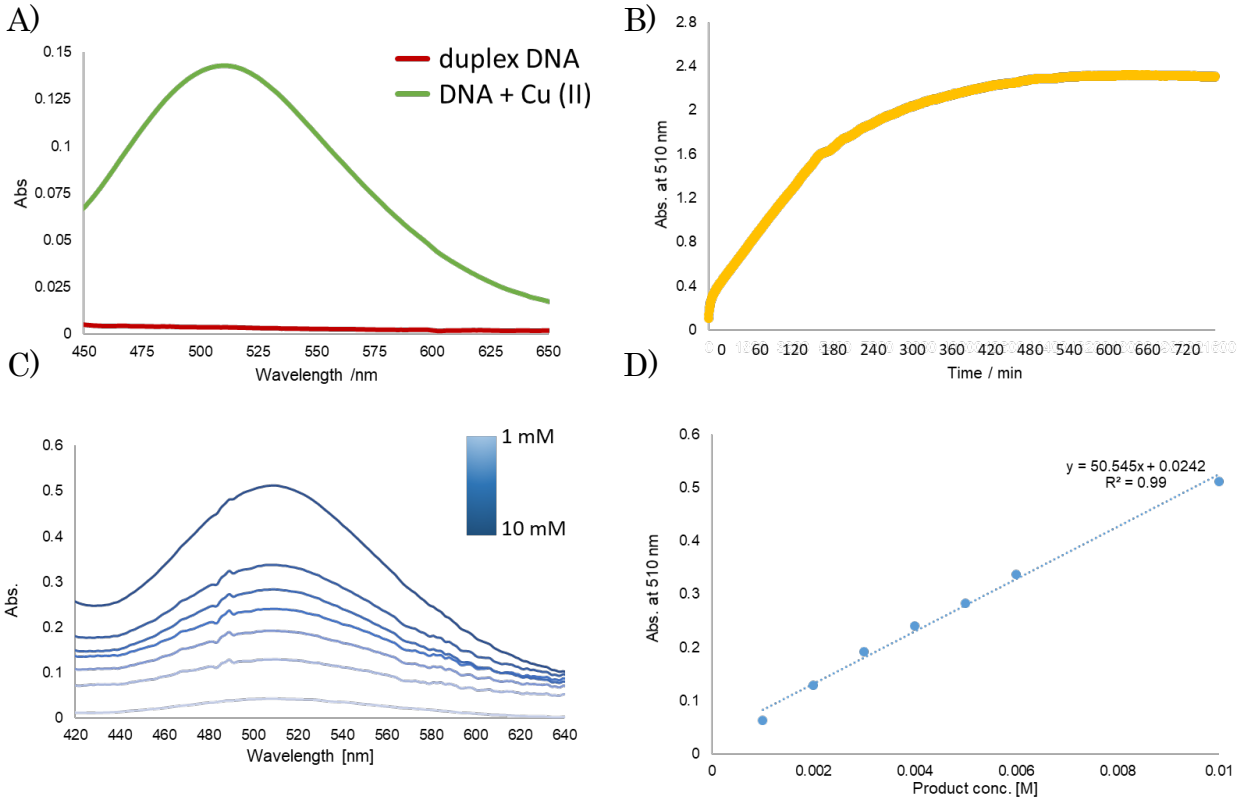


Figure S3. UV spectra of redox reactions with duplex-**HisHis** under various conditions. A) target detection in the presence and absence of copper ions, B) reaction monitoring at 510 nm for 12 hr, C) target detection in various concentration of purified product, D) titration graph of C)

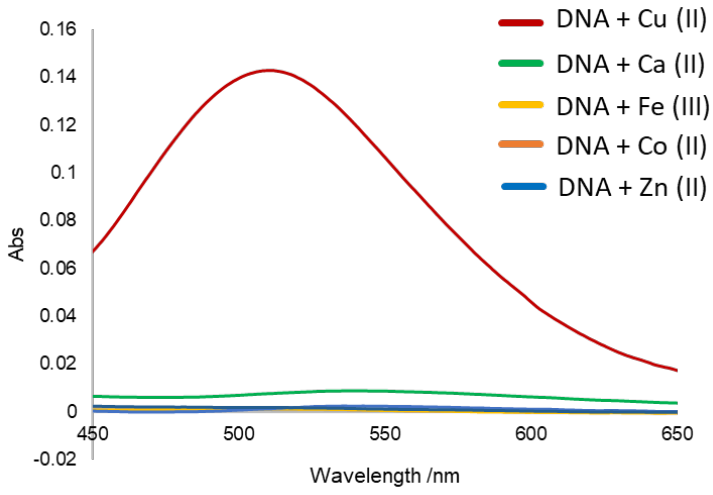


Figure S4. UV spectra of redox reactions with duplex-**HisHis** under various metal ions

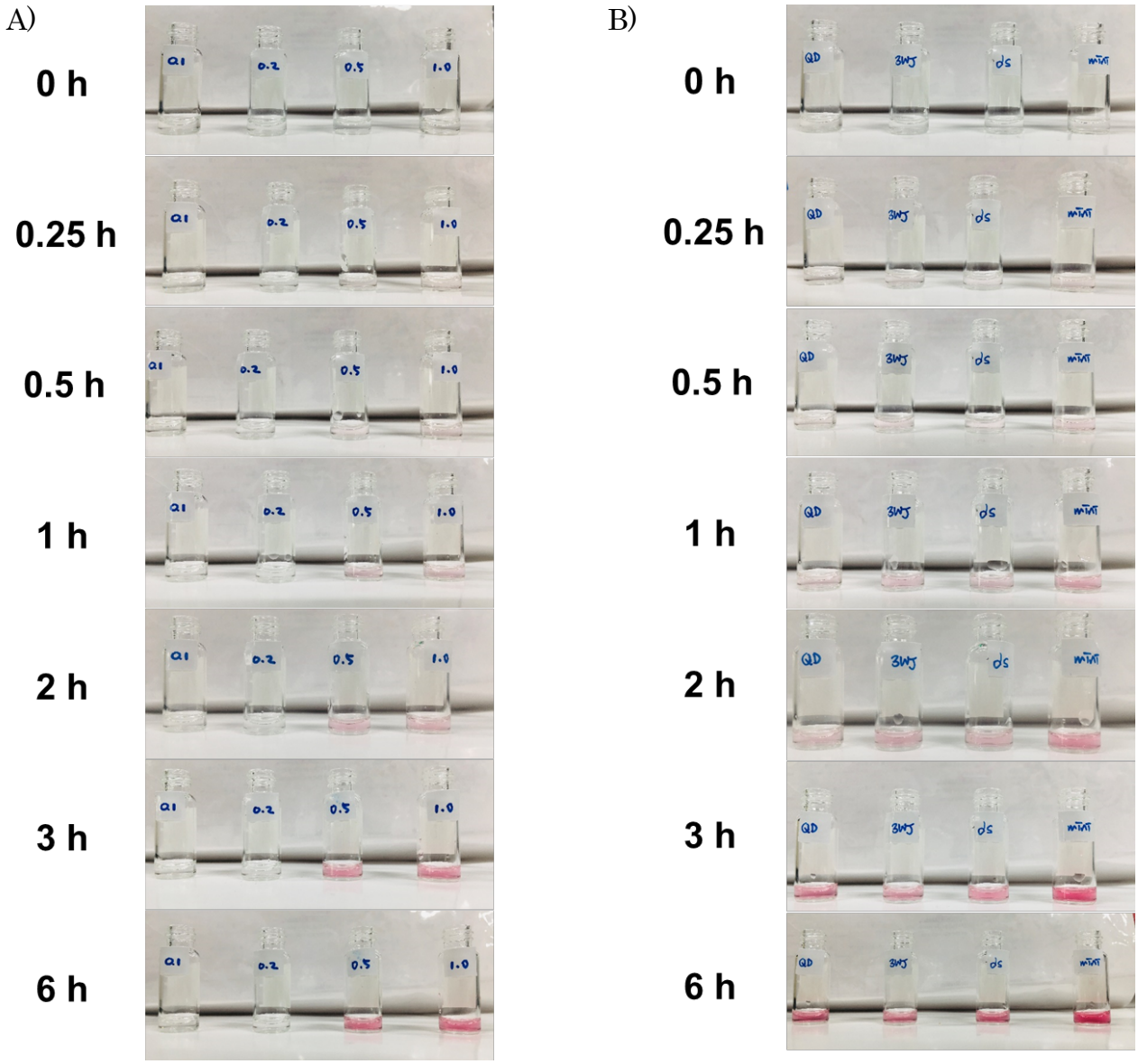


Figure S5. Reaction profiling with naked eyes. A) Observation of reaction progress catalyzed by various concentration of DNAzymes (from left, 0.1 mol%, 0.2 mol%, 0.5 mol%, and 1.0 mol%) during 6 hr, B) Observation of reaction progress catalyzed by various structure of DNAzymes (from left, QD-**HisHis**, 3WJ-**HisHis**, duplex-**HisHis**, and interG4-**HisHis**) during 6 hr.

Computational Work

Molecular modeling was carried out using the DS (Discovery Studio Client 2019) software package. The site-specific ANH-modification has conducted based on previously reported crystal structures (PDB ID: 2X88, 1DRG, 139D, 7CV3). We adopted 2X88 for laccase structure, 1DRG for three-way junction structure, 139D for intermolecular G-quadruplex, and 7CV3 for quadruplex-duplex hybrid structure, respectively. Sequence mutation has been performed for revising sequences of crystal sequences to synthesized sequences. Then duplex structure was built up from DS software. The building block of **His**-ANH was prepared and minimized (RMS gradient: 0.001) to obtain the optimal structure. The resulted **His**-ANH molecule was connected to the designated sequence of DNA structures. Minimizations were operated for each model with CHARM force field parameters. The obtained structures were shown as Figure S6-S9.

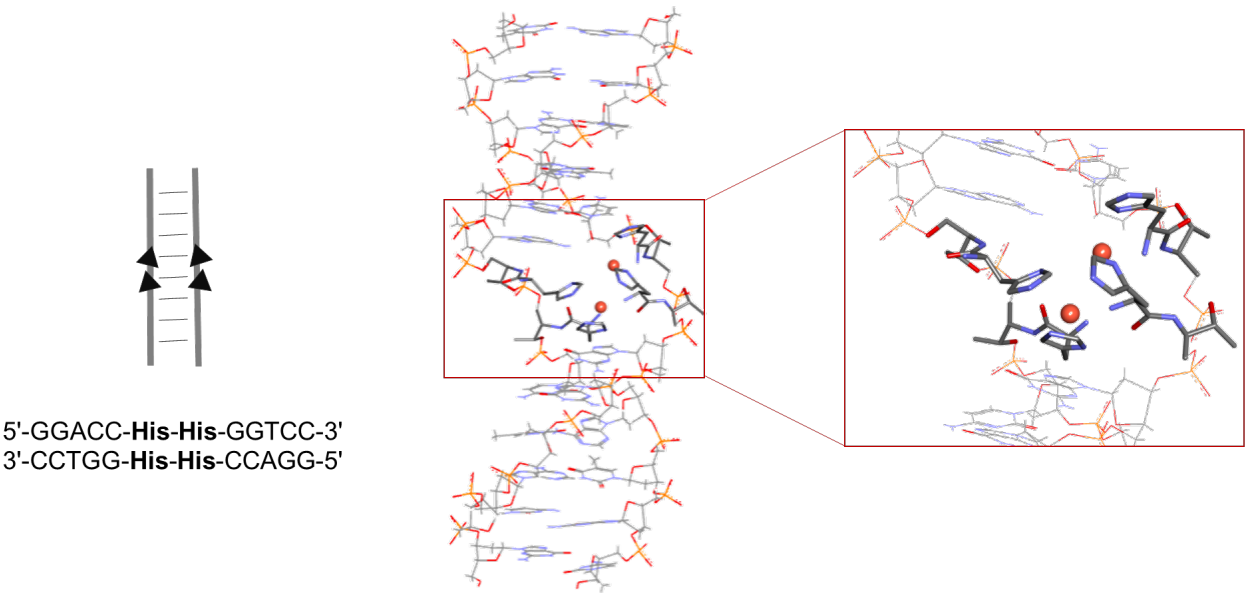


Figure S6. Energy minimized structure of duplex-**HisHis** (schematic illustration, overall structure, and magnified image of active site)

* Bold line structures indicate **His**-ANH, and copper ions are colored as reddish-brown ball

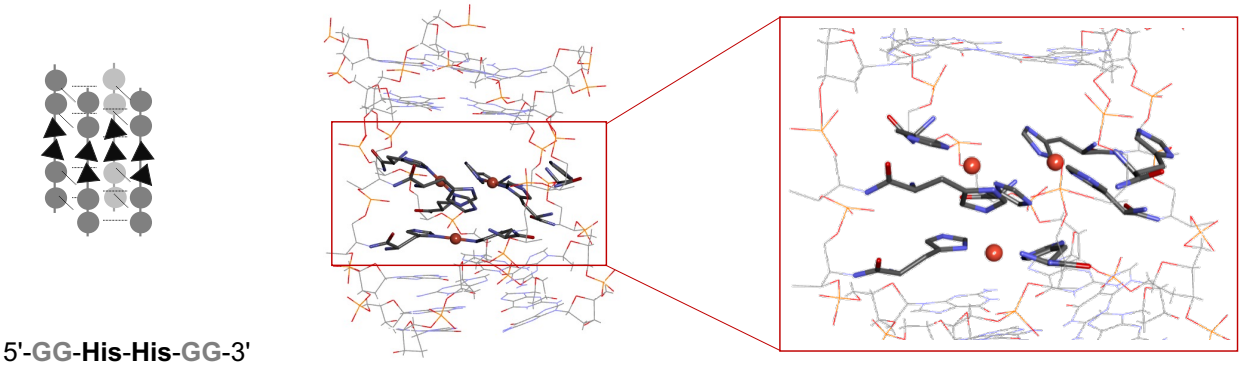


Figure S7. Energy minimized structure of interG4-**HisHis** (schematic illustration, overall structure, and magnified image of active site)

* Bold line structures indicate **His**-ANH, and copper ions are colored as reddish-brown ball

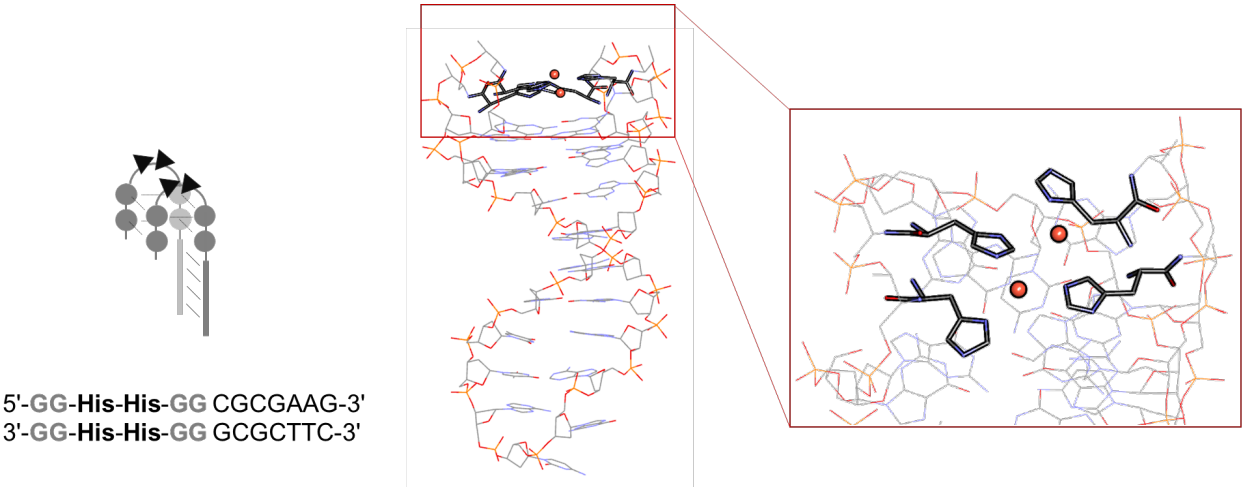


Figure S8. Energy minimized structure of QD-**HisHis** (schematic illustration, overall structure, and magnified image of active site)

* Bold line structures indicate **His**-ANH, and copper ions are colored as reddish-brown ball

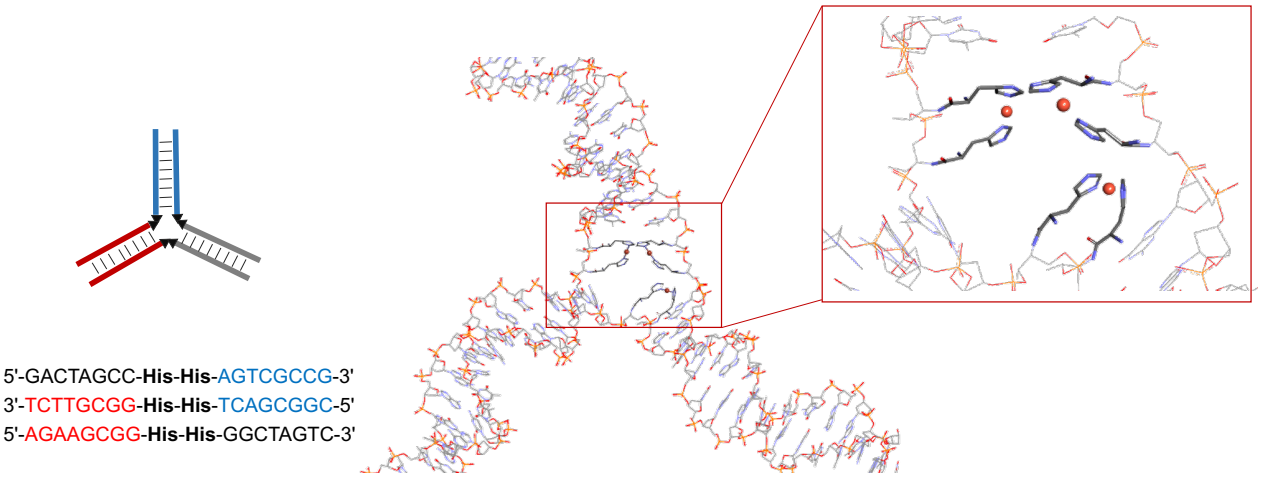


Figure S9. Energy minimized structure of 3WJ-**HisHis** (schematic illustration, overall structure, and magnified image of active site)

* Bold line structures indicate **His**-ANH, and copper ions are colored as reddish-brown ball

References

S1) Park, S.; Matsui, H.; Fukumoto, K.; Yum, J.H.; Sugiyama, H. *RSC Adv.,* **2020**, *10,* 9717−9722.

S2) Yum, J.H.; Ishizuka, T.; Fukumoto, K.; Hori, D.; Bao, H.L.; Xu, Y.; Sugiyama, H.; Park, S. *ACS Biomat. Sci. Eng.* **2021**, *7*, 1338−1343.