Electronic Supplementary Material (ESI) for Chemical Communications.

Designed Supramolecular Oxidase-Mimetic Catalyst for Colorimetric DNA Detection

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Materials

Table S1. Materials and regents

Materials	Abbreviation	CAS	Company
Fmoc-Lysine-OH·HCl	Fmoc-K HCl	139262-23-0	Mreda
Fmoc-Histidine-OH	Fmoc-H	116611-64-4	Aladdin
Fmoc-Arginine-OH	Fmoc-R	91000-69-0	Aladdin
3,3',5,5'-Tetramethylbenzidine	TMB	54827-17-7	Aladdin
Copper sulfate pentahydrate	$CuSO_4 \cdot 5H_2O$	7758-99-8	Aladdin
Magnesium sulfate heptahydrate	$MgSO_4 \cdot 7H_2O$	10034-99-8	Mreda

Water was deionized using a Milli-Q system(≥18.25 $M\Omega \cdot cm^{-1}$). Fmoc-K, Fmoc-H, Fmoc-R were dissolved in ultrapure water in the presence of 0.1 M hydrochloric acid. The solutions were kept for over two weeks priror to further use.

Table S2. DNA sequence

Abbreviation	sequence
a	AGTTCAGTTCAGTTCAGTTCAGTTCAGTTC
b	GAACTGAACTGAACTGAACTGAACTGAACT
c	AGTTCAGTTCAGTTCAGTTCAGTTCAGTTCAGTTCAGTTCAGTTCAGTTCAGTTC
d	GAACTGAACTGAACTGAACTGAACTGAACTGAACTGAACTGAACTGAACT
e	GTAGCGTGCGAAAGCGAAGCGTAGCGACTC
f	GAGTCGCTACGCTTCGCTTTCGCACGCTAC

g	GTAGCGTGCGAAAGCGAAGCGTAGCGACTCGTGCGAAAGCGAAGCGTAGCAGCTC
h	GAGCTGCTACGCTTCGCTTTCGCACGAGTCGCTACGCTTCGCTTTCGCACGCTAC
i	GAATACGAACTAGAACTAATGCAACTATAG
j	CTATAGTTGCATTAGTTCTAGTTCGTATTC
k	CTATAGTTGCATAAGTTCAAGATCGTATTC
l	CTATAGTAGCATAAGTACAAGATCGTATTC
m	CTAAAGTAGCATAAGTACAAGATCGAATTC
n	TGCCGGGCGTCCGCGCCGTAGGTGCATA
o	TATGCACCTACGGCGCGGACGCCCGGCAAGACGTGTATGCCGGGCGTCCGCGCCGT
p	TGCCGGGCGTCCGCGCCGTAGGTGCATAACGGCGCGGACGCCCGGCATACACGTCT
q	TGCCGGGCGTCCGCGCCGTAGTGAGTCA
r	AAAAAAAAAAAAAAAAAAA
s	AAAAAAAAAGAAAAAAAAA
t	AAAAAAAAAGAAAAAAAAG
u	GAAAAAAAAGAAAAAAAAG
v	GAAAGAAAAGAAAAGAAAAG
w	GAAGAAGAAGAAGAAGAGAG

Water was deionized using a Milli-Q system(≥18.25 MΩ·cm^{-1}). DNA is dissolved in water at a concentration of 100 μM. The concentration is determined by UV-Vis spectroscopy.

Methods

Instruments

UV-Vis absorption spectra were recorded using a UV-2600 spectrometer equipped with a temperature-control accessory (Shimadzu).

Fluorescent emission spectra were recorded using a G9800A fluorescence spectrophotometer with a temperature-control accessory (Agilent Technologies).

TEM characterization was conducted on an Hitachi 7800 microscope in bright-field mode at 80 kV.

CD spectra were recorded with a J-815 spectropolarimeter (Jasco) under the following conditions: optical path, 0.5 mm, bandwidth, 10 nm, scan speed, 50nm/min.

EPR measurements were conducted on an ELEXSYS-II E500 EPR spectrometer with a low-temperature accessory (Bruker BioSpin).

Activity assay

The amino acid amphiphiles (Fmoc-K; Fmoc-H; Fmoc-R) were dissolved in ultrapure water to make 100 mM stock solutions and stored for over two weeks prior to use. Then, the amino acid amphiphiles, DNA and copper ions were added to acetate buffer solution (50 mM, pH = 6.00) at the required concentrations, followed by incubation at room temperature for 4 h. With TMB as substrates, the reactions were performed at acetate buffer solution. Time-dependent absorbance changes were recorded, and the data were used to calculate the initial catalytic velocity (V_i) and apparent kinetic parameters.

Fluorescence assay

Effect of DNA on Fmoc-K stacking. The amino acid amphiphiles and DNA were added to ultrapure water at the required concentrations, followed by incubation at room temperature for 4 h. Experiment conditions: excitation wavelength, 320 nm, emission wavelength, 340 nm - 620 nm, scan rate, 600 nm/min.

Calculate the binding constant. The amino acid amphiphiles, DNA and copper were added to ultrapure water at the required concentrations. Experiment conditions: excitation wavelength, 492 nm, emission wavelength, 510 nm - 600 nm, scan rate, 600 nm/min.

Continuous-Wave Electron Paramagnetic Resonance (CW-EPR) Spectroscopy.

Samples were transferred to EPR tubes and frozen by liquid nitrogen atmosphere for use in EPR experiments. CW-EPR spectra were collected by using a Bruker ELEXSYS-II E500 CW-EPR spectrometer equipped with Air Products cryostat and temperature controller modified for nitrogen gas flow sample cooling. Spectra were acquired under the following condition: Microwave frequency, 9.39 GHz; microwave power, 10 mW, modulation amplitude, 2 Gauss; modulation frequency. 100 kHz; sweep time, 120 s; temperature, 77 K. A minimum of 5 scans were recorded and averaged for each spectrum. All spectra simulations were used by hyperfinespectrum 1.0.0.

Calculation of detection limits

$LOD = 3.3\delta/S$

where S is the slope of the corresponding calibration curve and δ is the standard deviation of the blank signals.

Calculation of activation energy

$$\ln K = -\frac{E_a}{RT} + \ln A$$

$$K = \frac{V_i}{[Cu^{2+}][TMB]}$$

where E_a is the activation energy.

Calculation of binding constant

$$\frac{F_0}{F} = 1 + K_b[Q]$$

where F_0 is the fluorescence intensity with zero copper ion concentration and F is the fluorescence intensity at the copper ion concentration of Q. K_b is the binding constant.

Hybridization chain reaction (HCR)

Gel electrophoresis. The DNA was heated to 95°C for 2 minutes, followed by a 1-hour cooling period at room temperature before use. A 1% agarose gel (refer to Figure S16) was prepared using 1×TBE buffer, which included 10 mM MgSO4. The agarose gels were electrophoresed at 150 V for 60 minutes and visualized under UV light. For the HCR reactions (refer to Figure S16), stock solutions of **n** (or **q**), **o**, and **p** were appropriately diluted to their final concentrations. Subsequently, 9 μL of each component were combined to yield a total reaction volume of 27 μL. These reactions were then left to incubate at room temperature for 4 hours prior to loading 20 μL of each resulting product onto a gel.

Activity assay. The amino acid amphiphiles (Fmoc-K, Fmoc-H, or Fmoc-R) were dissolved in ultrapure water to prepare 100 mM stock solutions, which were stored for a minimum of two weeks prior to use. The DNA was heated to 95°C for 2 minutes and subsequently allowed to cool to room temperature over the course of 1 hour before application. For the reaction (please refer to Figure S17), the stock solutions of **n** (or **q**), **o**, and **q** were sequentially introduced into the reaction buffer and then appropriately diluted to achieve their final concentrations. Following a 4-hour incubation at room temperature, the amino acid amphiphiles and copper ions were introduced to attain the required concentration, after which they were incubated at room temperature for another 4 hours. Time-dependent absorbance changes at 652 nm were recorded, and

the data were used to obtain the initial catalytic velocity (V_i).

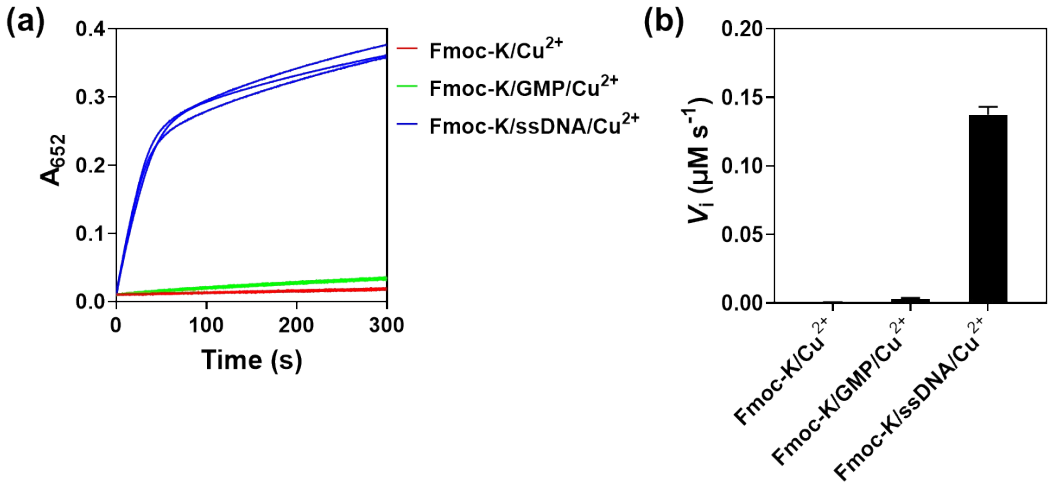

Fig. S1. (a) Time-dependent absorbance changes at 652 nm and (b) oxidation rates of TMB catalyzed by Fmoc-K/Cu^{2+} or Fmoc-K/GMP/Cu^{2+} or Fmoc-K/ssDNA/Cu^{2+} complex. [Fmoc-K] = 1 mM, [DNA **c**] = 0.1 μM, [GMP] = 5.5 μM, [Mg^{2+}] = 10 mM, [Cu^{2+}] = 5 μM, [Acetate buffer] = 50 mM, pH = 6.0.

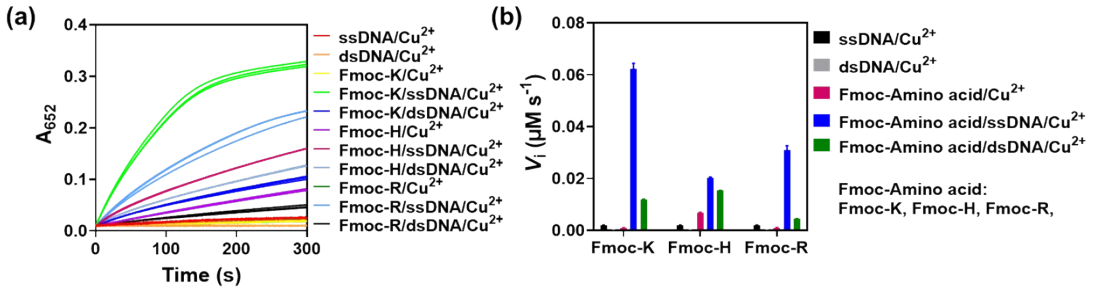

Fig. S2. (a) Time-dependent absorbance changes at 652 nm and (b) oxidation rates of TMB catalyzed by Fmoc-K or Fmoc-H or Fmoc-R /DNA/Cu^{2+} complex. [Fmoc-K] = 1 mM, [DNA **c**] = 0.1 μM, [DNA **d**] = 0.1 μM, [Mg^{2+}] = 10 mM, [Cu^{2+}] = 5 μM, [Acetate buffer] = 50 mM, pH = 6.0.

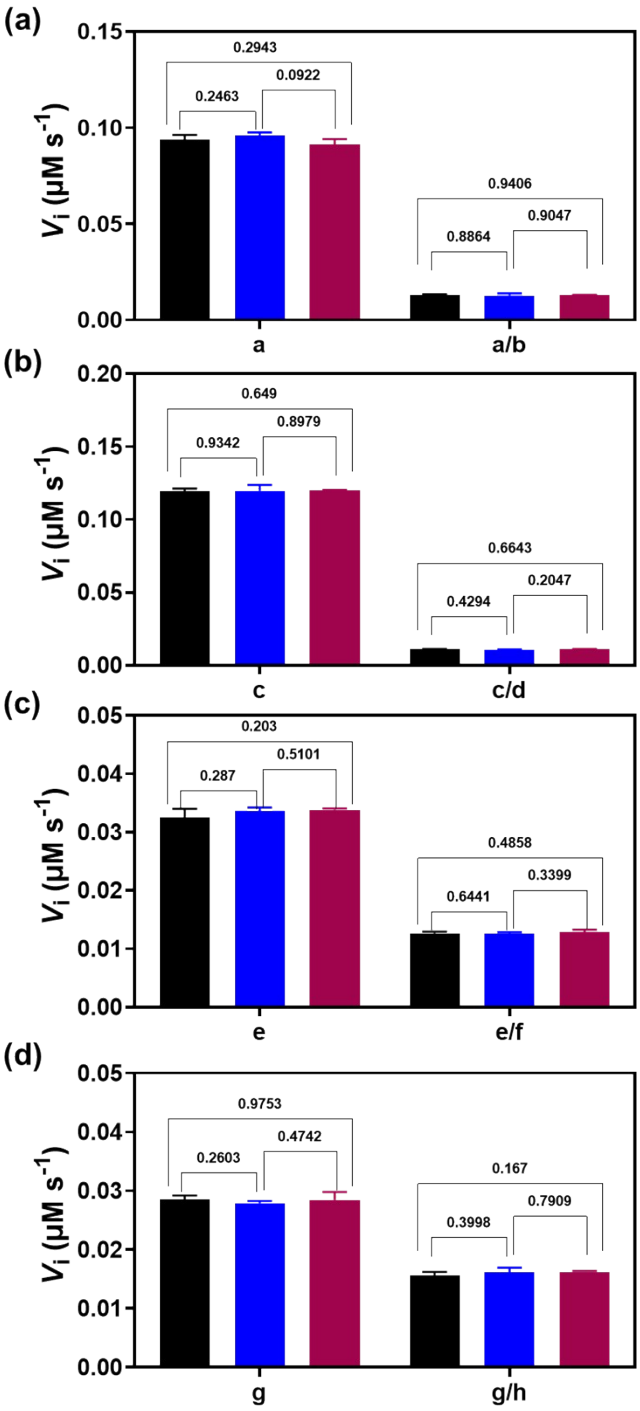

Fig. S3. Three samples of Fmoc-K/Cu^{2+} complexes containing (a) DNA a, a/b; (b) c, c/d; (c) e, e/f; (d) g, g/h were prepared independently, for calculation of *p*-value using *t*-test. [Fmoc-K] = 1 mM, [DNA] = 0.1 μM, [Mg^{2+}] = 10 mM, [Cu^{2+}] = 5 μM, [Acetate buffer] = 50 mM, pH = 6.0. The *p*-value was greater than the commonly chosen threshold of 0.05, suggesting the activity repeated consistently without significant variation. This means that the observed results

are not considered statistically significant.

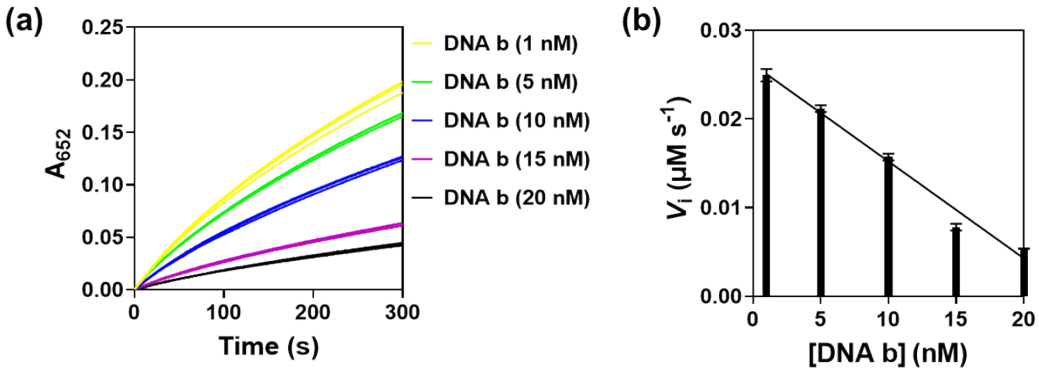

Fig. S4. (a) Time-dependent absorbance changes at 652 nm and (b) oxidation rates of TMB catalyzed by Fmoc-K/DNA (**a**/**b**)/Cu^{2+} complex at various DNA **b** concentrations. Used to calculate the detection limit of DNA **b**. [Fmoc-K] = 1 mM, [DNA **a**] = 0.02 μM, [Cu^{2+}] = 5 μM, [Mg^{2+}] = 10 mM, [Acetate buffer] = 50 mM, pH = 6.0.

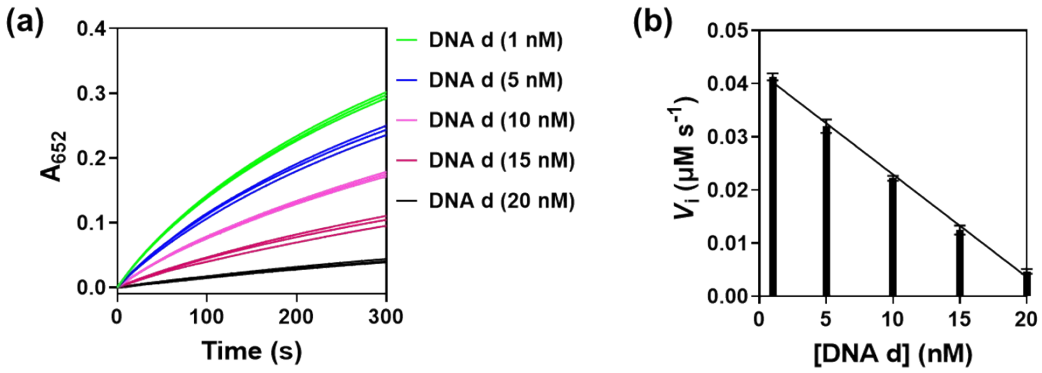

Fig. S5. (a) Time-dependent absorbance changes at 652 nm and (b) oxidation rates of TMB catalyzed by Fmoc-K/DNA (**c**/**d**)/Cu^{2+} complex at various DNA **d** concentrations. Used to calculate the detection limit of DNA **d**. [Fmoc-K] = 1 mM, [DNA **c**] = 0.02 μM, [Cu^{2+}] = 5 μM, [Mg^{2+}] = 10 mM, [Acetate buffer] = 50 mM, pH = 6.0.

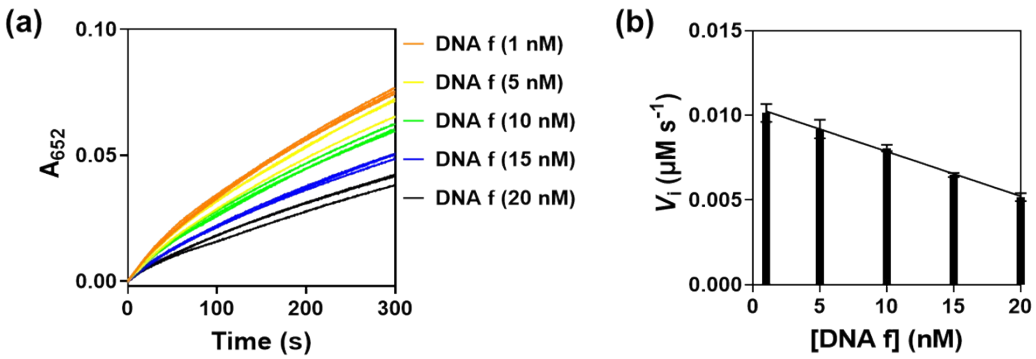

Fig. S6. (a) Time-dependent absorbance changes at 652 nm and (b) oxidation rates of TMB catalyzed by Fmoc-K/DNA (**e**/**f**)/Cu^{2+} complex at various DNA **f** concentrations. Used to calculate the detection limit of DNA **f**. [Fmoc-K] = 1 mM, [DNA **e**] = 0.02 μM, [Cu^{2+}] = 5 μM, [Mg^{2+}] = 10 mM, [Acetate buffer] = 50 mM, pH = 6.0.

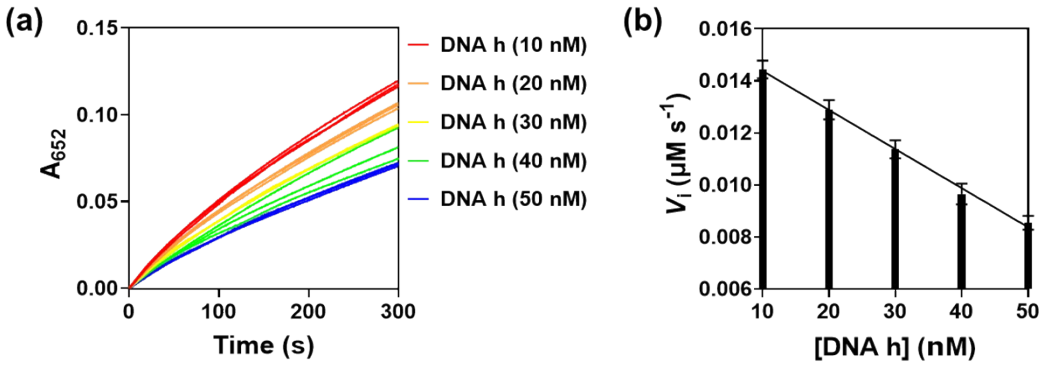

Fig. S7. (a) Time-dependent absorbance changes at 652 nm and (b) oxidation rates of TMB catalyzed by Fmoc-K/DNA (**g**/**h**)/Cu^{2+} complex at various DNA **h** concentrations. Used to calculate the detection limit of DNA **h**. [Fmoc-K] = 1 mM, [DNA **g**] = 0.05 μM, [Cu^{2+}] = 5 μM, [Mg^{2+}] = 10 mM, [Acetate buffer] = 50 mM, pH = 6.0.

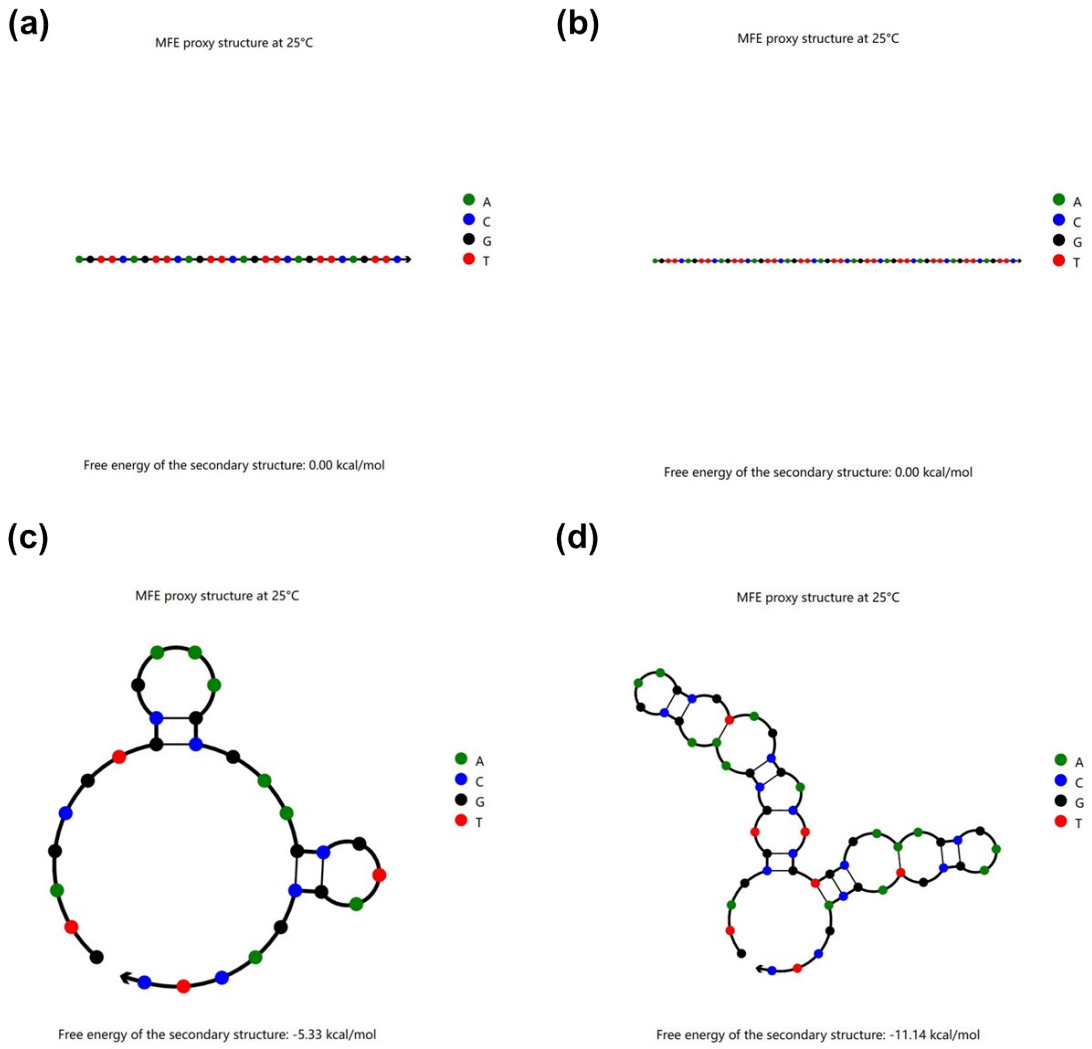

Fig. S8. The simulated structure of DNA (a) **a** (b) **c** (c) **e** and (d) **g**.

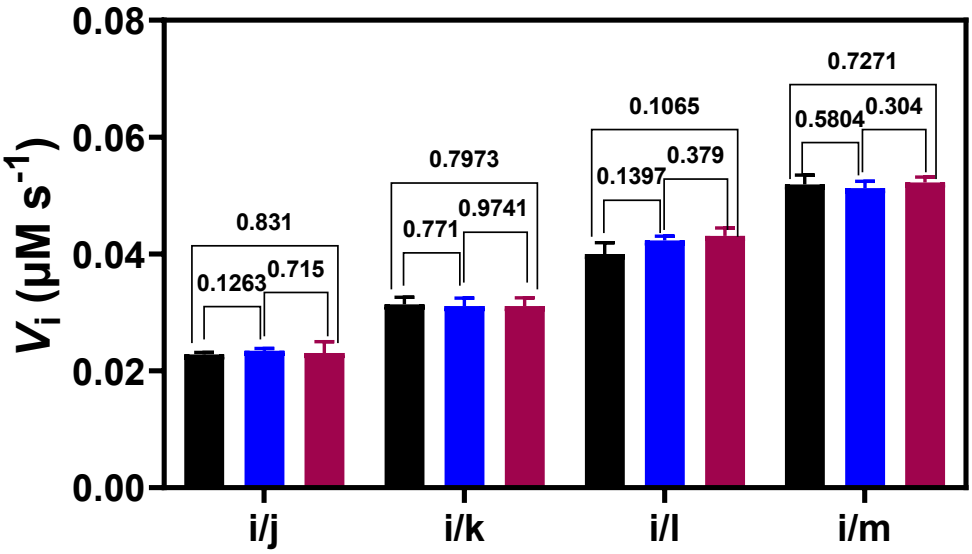

Fig. S9. Three samples of Fmoc-K/Cu^{2+} complexes containing DNA i/j, i/k, i/l, i/m were prepared independently, for calculation of *p*-value using *t*-test. The *p*-value was greater than the commonly chosen threshold of 0.05, suggesting the activity repeated consistently without significant variation. This means that the observed results are not considered statistically significant. [Fmoc-K] = 1 mM, [DNA] = 0.1 μM, [Cu^{2+}] = 5 μM, [Acetate buffer] = 50 mM, pH = 6.0.

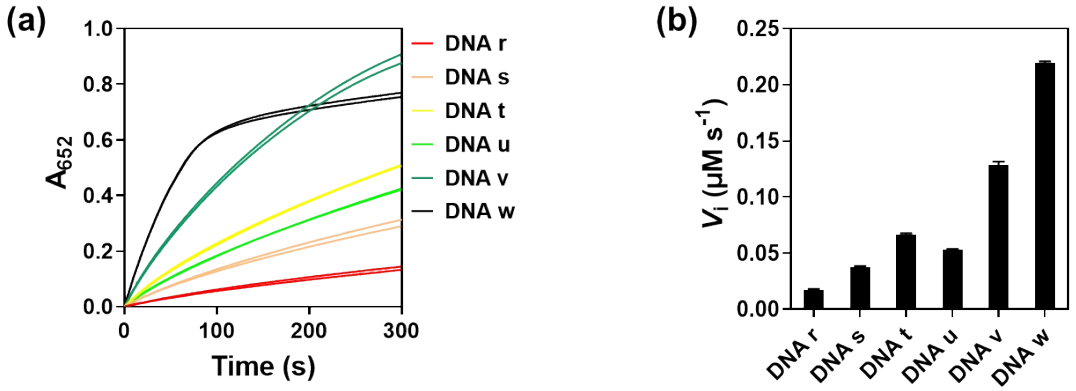

Fig. S10. (a) Time-dependent absorbance changes at 652 nm and (b) oxidation rates of TMB catalyzed by Fmoc-K/DNA/Cu^{2+} complex at various DNA sequences. [Fmoc-K] = 1 mM, [DNA] = 1 μM, [Cu^{2+}] = 5 μM, [Mg^{2+}] = 10 mM, [Acetate buffer] = 50 mM, pH = 6.0.

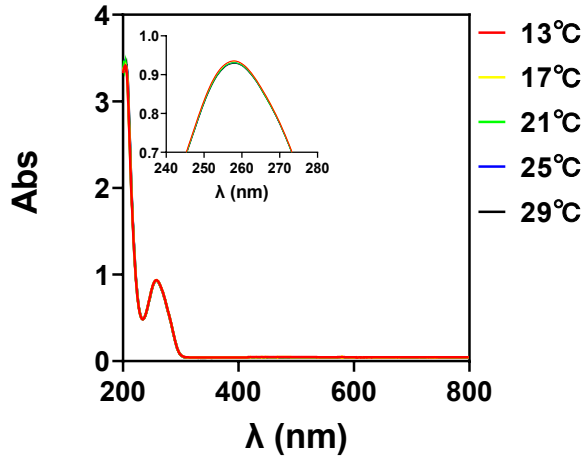

Fig. S11. Uv-vis absorption spectra of Fmoc-K/dsDNA/Cu^{2+} complex at different temperatures. [Fmoc-K] = 1 mM, [DNA **c**] = 0.1 μM, [DNA **d**] = 0.1 μM, [Cu^{2+}] = 5 μM, [Mg^{2+}] = 10 mM, [Acetate buffer] = 50 mM, pH = 6.00.

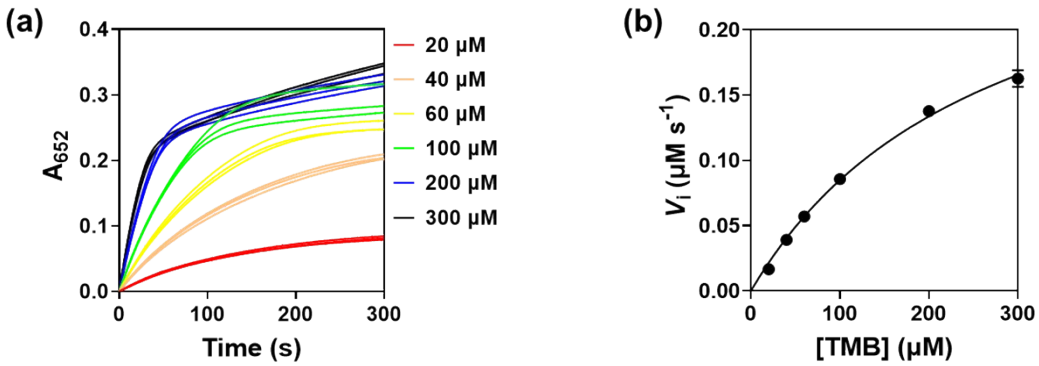

Fig. S12. (a) Time-dependent absorbance changes at 652 nm and (b) catalytic kinetics calculation of Fmoc-K/ssDNA/Cu^{2+} complex at different TMB concentrations. [Fmoc-K] = 1 mM, [DNA **c**] = 0.1 μM, [Cu^{2+}] = 5 μM, [Mg^{2+}] = 10 mM, [Acetate buffer] = 50 mM, pH = 6.0.

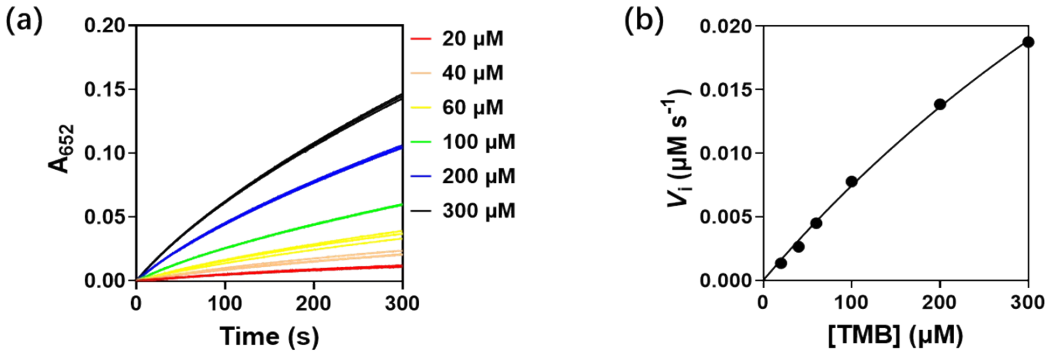

Fig. S13. (a) Time-dependent absorbance changes at 652 nm and (b) catalytic kinetics calculation of Fmoc-K/dsDNA/Cu^{2+} complex at different TMB concentrations. [Fmoc-K] = 1 mM, [DNA **c**] = 0.1 μM, [DNA **d**] = 0.1 μM, [Cu^{2+}] = 5 μM, [Mg^{2+}] = 10 mM, [Acetate buffer] = 50 mM, pH = 6.0.

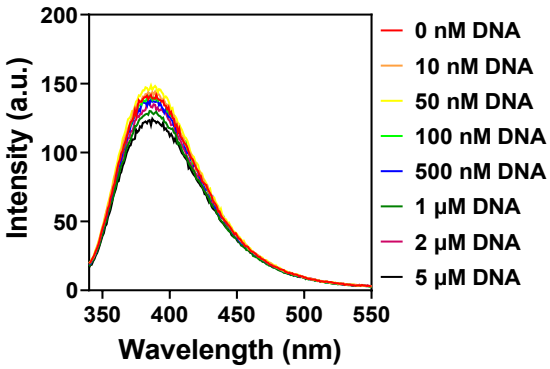

Fig. S14. Fluorescence spectra of Fmoc-K at various dsDNA **c**/**d** concentrations. [Fmoc-K] = 1 mM, [Cu^{2+}] = 5 μM, [Mg^{2+}] = 10 mM, [Acetate buffer] = 50 mM, pH = 6.00.

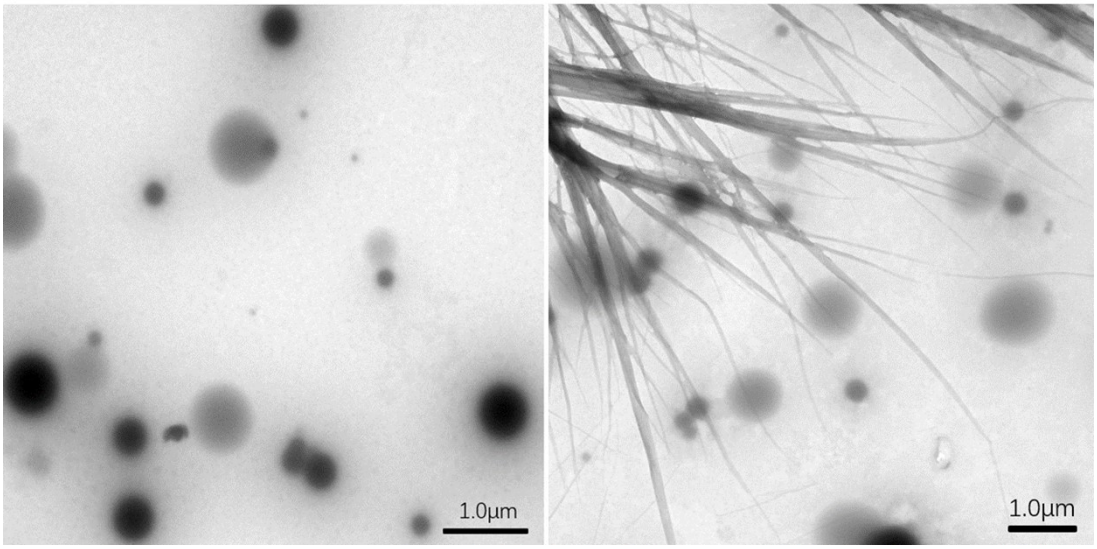

Fig. S15. Transmission electron microscopy of Fmoc-K/ssDNA/Cu^{2+} complex. [Fmoc-K] = 1 mM, [DNA **c**] = 0.1 μM, [Cu^{2+}] = 5 μM, [Mg^{2+}] = 1 mM.

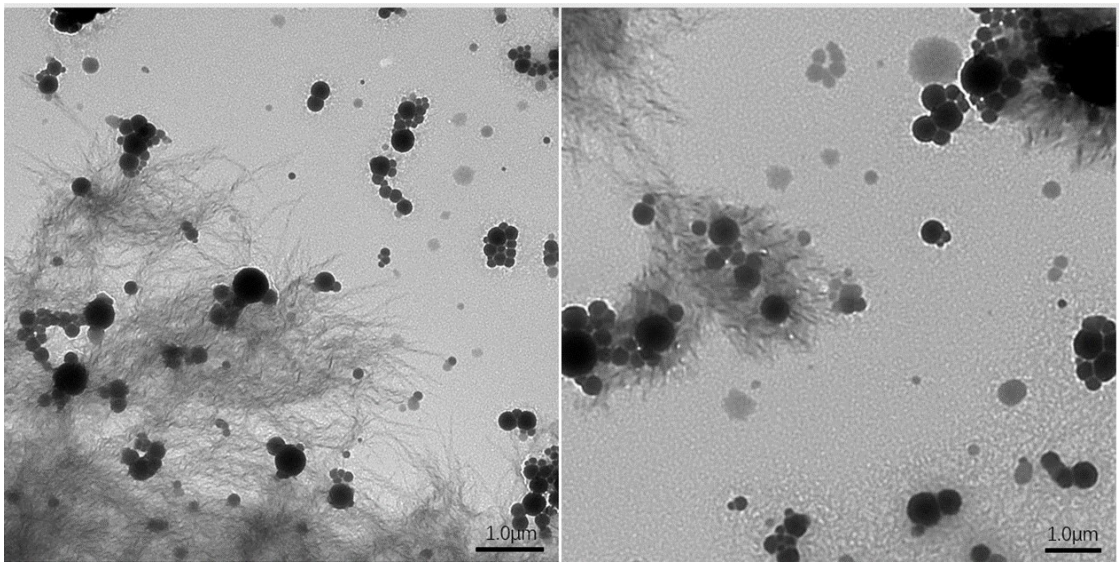

Fig. S16. Transmission electron microscopy of Fmoc-K/dsDNA/Cu^{2+} complex. [Fmoc-K] = 1 mM, [DNA **c**] = 0.1 μM, [DNA **d**] = 0.1 μM, [Cu^{2+}] = 5 μM, [Mg^{2+}] = 1 mM.

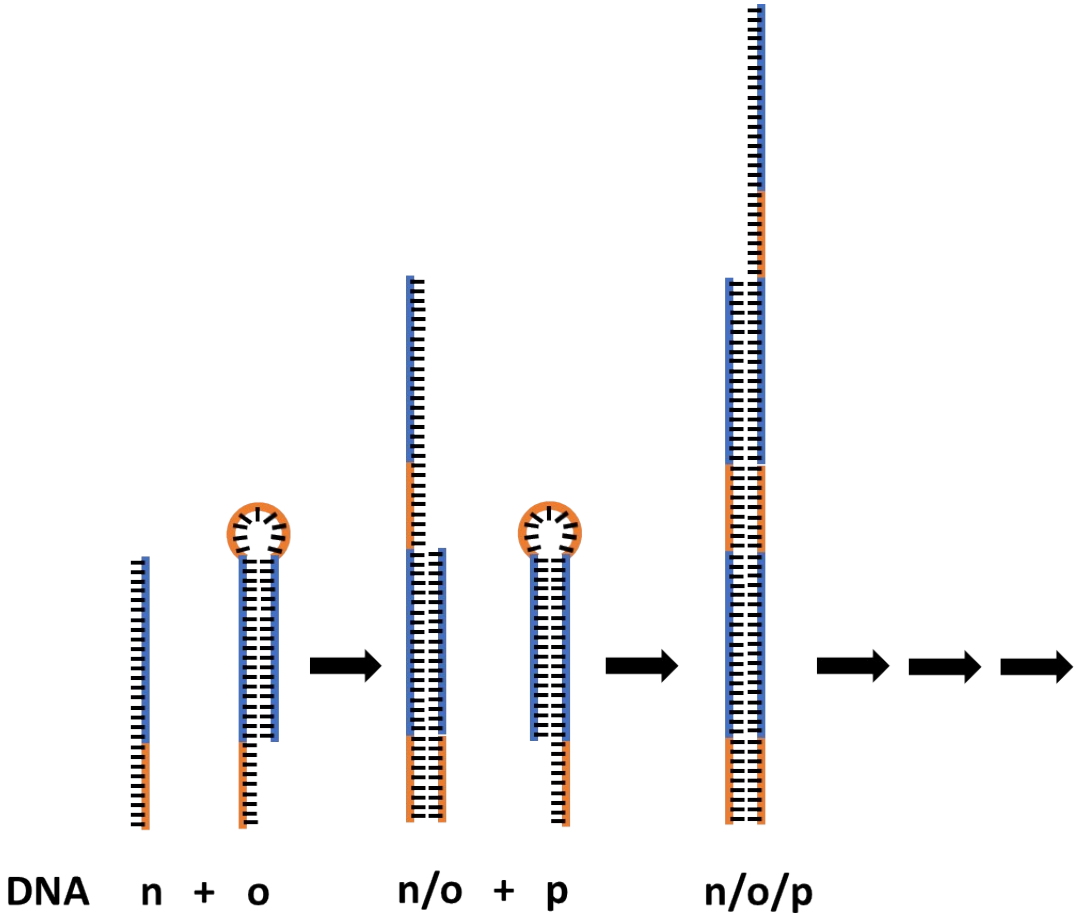

Fig. S17. Secondary structure schematic of HCR function.

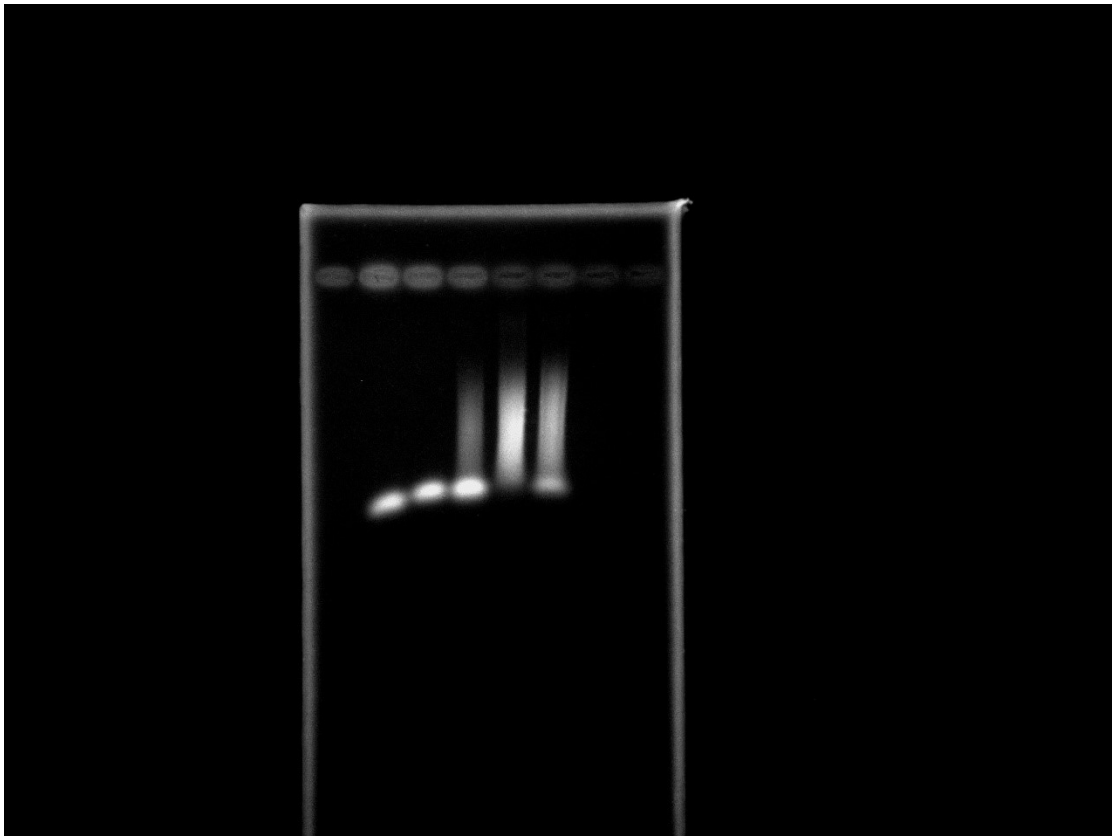

Fig. S18.The 1% agarose gel electrophoresis (1×TBE/10mM $MgSO_4$). Sample: (1) DNA **o**, (2) DNA **p**, (3) DNA **o**/**p**, (4) DNA **n**/**o**/**p**, (5) DNA **q**/**o**/**p**. [DNA **o**] = [DNA **p**] = 3 μM, [DNA **n**] = [DNA **q**] = 0.3 μM, [Mg^{2+}] = 10 mM, [Acetate buffer] = 50 mM, pH = 6.0.

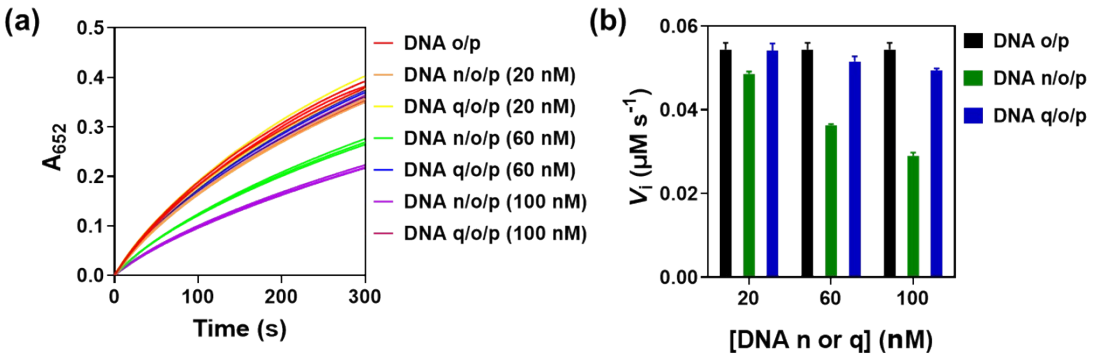

Fig. S19. (a) Time-dependent absorbance changes at 652 nm and (b) oxidation rates of TMB catalyzed by Fmoc-K/DNA/Cu^{2+} complex at various DNA **n** or **q** concentrations. [Fmoc-K] = 1 mM, [Cu^{2+}] = 5 μM, [DNA **o**] = [DNA **p**] = 1 μM, [Mg^{2+}] = 10 mM, [Acetate buffer] = 50 mM, pH = 6.0.

Table S3. Detection limits for DNA **b**, **d**, **f, h**

DNA	**b**	**d**	**f**	**h**
Number of bases	30	55	30	55
Number of CGs	12	22	18	33
Detection limits	0.4736	0.2687	1.9632	3.4517

Table S4. Kinetic parameters and activation energy

	V_{max} (μM s^{-1})	***K*$_m$ (μM)**	***k*cat (s^{-1})**	***k*cat/*K*m (μM^{-1} s^{-1})**	**activation energy (kJ mol^{-1})**
ssDNA	0.3148	270.9	0.06296	$2.3241X10^{-4}$	21.109
dsDNA	0.08125	990.9	0.01625	$1.6399X10^{-5}$	36.033