Electronic Supplementary Information

Nanoceria as a DNase I mimicking nanozyme

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Materials and Methods

Chemicals. All the DNA oligomers were purchased from Integrated DNA Technologies (IDT, Coralville, IA, USA), and their sequences and modifications are listed in **Table S1**. The DNase I and its reaction buffer (10×) was purchased from New England Biolabs. Nanoceria dispersion (catalog number: 289744, 20% dispersed in 2.5% acetic acid), and metal salts, and the other nanoparticles and their characterization were reported previously.[1, 2] Trisodium phosphate and ethylenediaminetetraacetic acid (EDTA) were from Mandel Scientific, Inc. (Guelph, Ontario, Canada). The nanoceria sample was washed three times using ultracentrifugation. Milli-Q water was used for all the experiments.

DNA cleavage by nanoceria. Detailed characterization of nanoceria used in this work was reported in our previous studies.[3, 4] Each DNA sample was dissolved in deionized water to a final concentration of 1.0 μM in the presence of nanoceria (100 μg/mL) under different temperatures (0-60^{o}C). The cleavage products were separated using 15% denaturing polyacrylamide gel electrophoresis (dPAGE) and analyzed using a ChemDoc MP imaging system (Bio-Rad, USA). Cleavage by other metal oxide nanoparticles was performed using the same protocol.

DNA cleavage by DNase I. Each DNA sample was dissolved with 1× DNase I reaction buffer to a final concentration of 1.0 μM in the presence of DNase I (100 U/mL) at 37°C. The cleavage products were separated using 15% denaturing polyacrylamide gel electrophoresis (dPAGE) and analyzed using a ChemDoc MP imaging system (Bio-Rad, USA).

Mass spectrometry analysis of DNA cleavage products. For mass spectrometry studies, non-FAM-labeled A_5 and G_5 DNA were used. Each DNA was reacted at 1.0 μM with 100 μg/mL of CeO_2 for 4 h at 60°C in a total volume of 5 mL. After the reaction, the cleavage products were further concentrated and desalted using a Sep-Pak C_{18} cartridge (eluted with CH_3OH/CH_3CN mixture, 9:1, v:v) and dried in a vacuum centrifuge instrument. The dried samples were then dissolved by 50 μL Milli-Q water for mass spectrometry analysis. High resolution mass spectrometry analysis was performed using a Thermo Scientific Q-Exactive Orbitrap mass spectrometer (Thermo Scientific, USA) coupled with an electrospray ionization (ESI) source. To help the cleavage products to enter the gas phase, ammonium acetate (0.01M) was added.

Fluorescence analysis. DNA adsorption by nanoceria was analyzed by monitoring the change of fluorescence intensity upon the adding of nanoceria. The initial concentration of DNA (FAM-A_{15}) was 1.0 μM. The fluorescence spectra of the FAM-A_{15} samples were acquired after adding different concentrations of nanoceria. All fluorescence analysis was performed using a Cary Eclipse fluorometer (Varian, USA) by exciting at 485 nm.

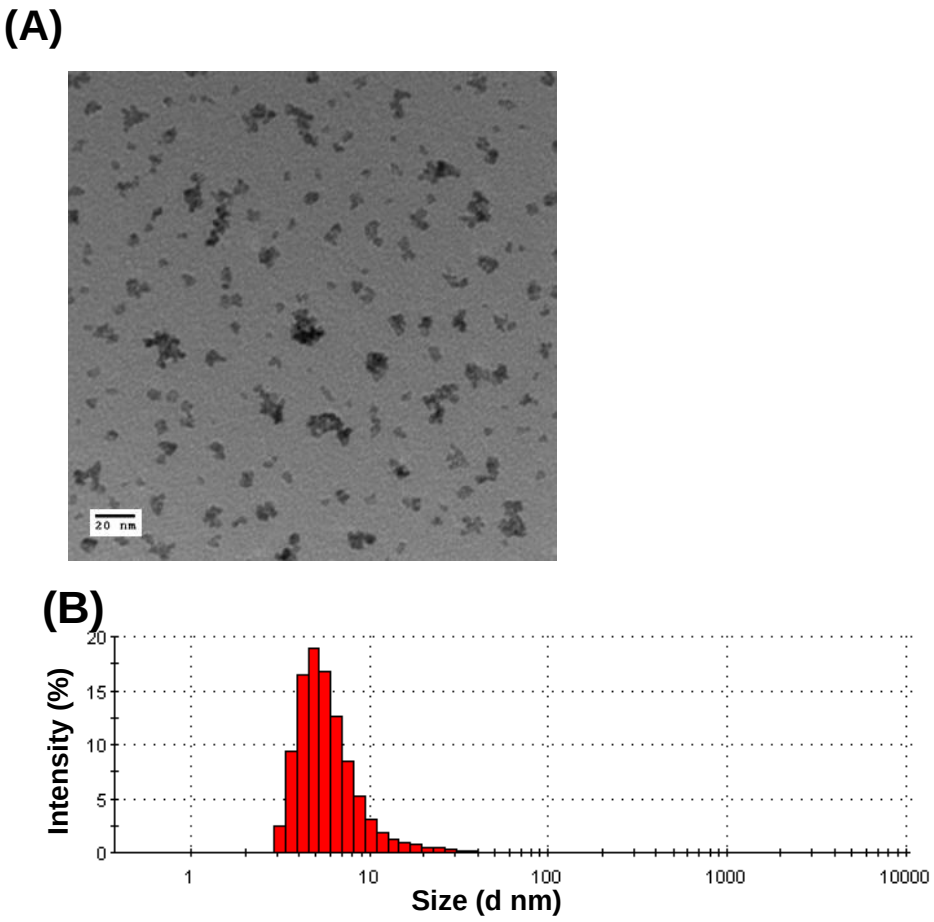


Fig. S1. (A) A TEM micrograph and (B) DLS size distribution of the CeO_2 nanoparticles used in this work. The average size was around 5 nm and slight aggregation was observed.

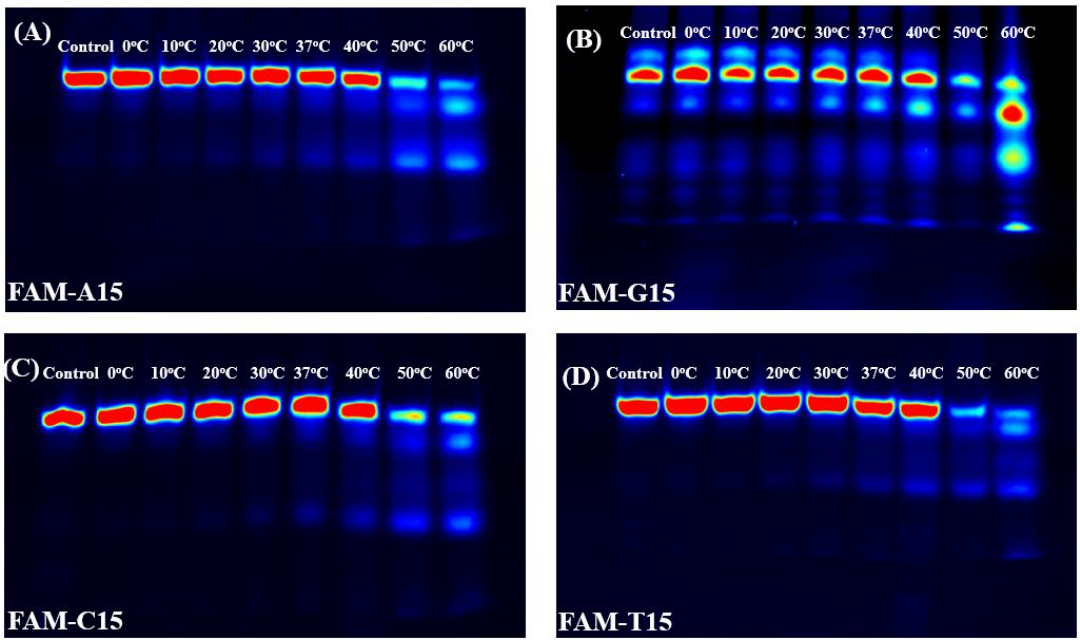


Fig. S2. Polyacrylamide gel electrophoresis analysis of DNA (1.0 μM) cleavage after incubated with nanoceria (100 μg/mL) at different temperatures (0-60°C) for 4 h. (A) FAM-A_{15}; (B) FAM-G_{15}; (C) FAM-C_{15}; (D) FAM-T_{15}. The cleavage yield increased with increasing temperature.

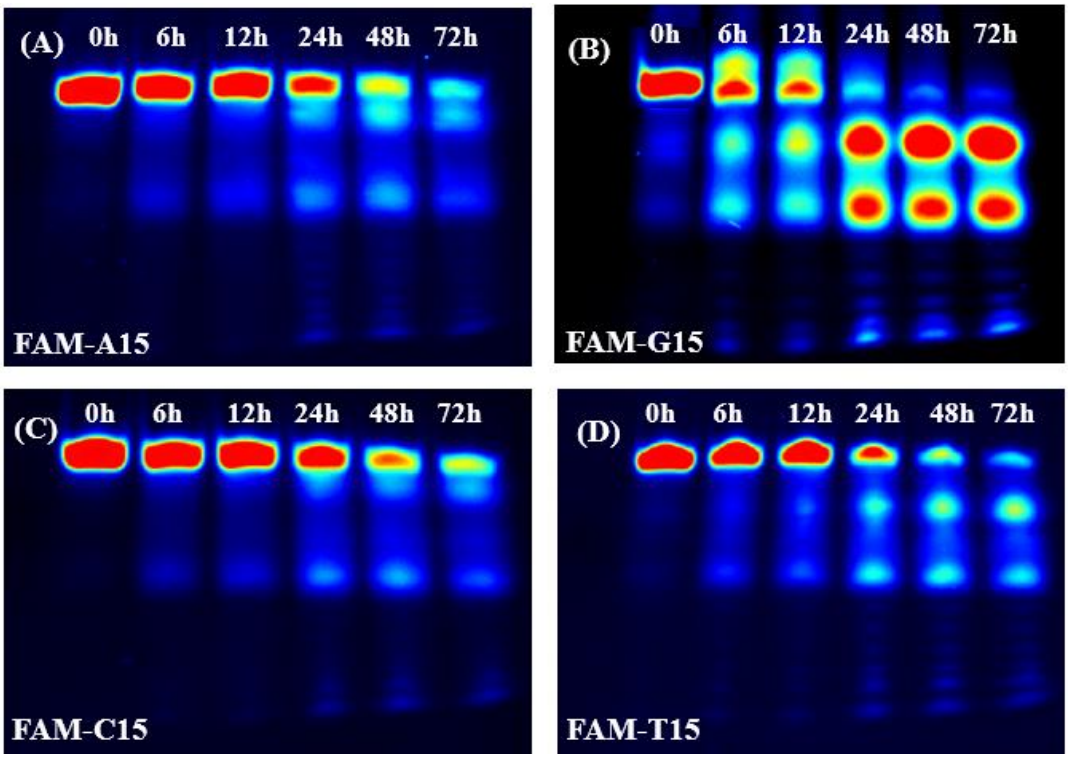


Fig. S3. Polyacrylamide gel electrophoresis analysis of DNA (1.0 μM) cleavage by nanoceria (100 μg/mL) at 37°C after 72 h incubation. (**A**) FAM-A_{15}; (**B**) FAM-G_{15}; (**C**) FAM-C_{15}; (**D**) FAM-T_{15}.

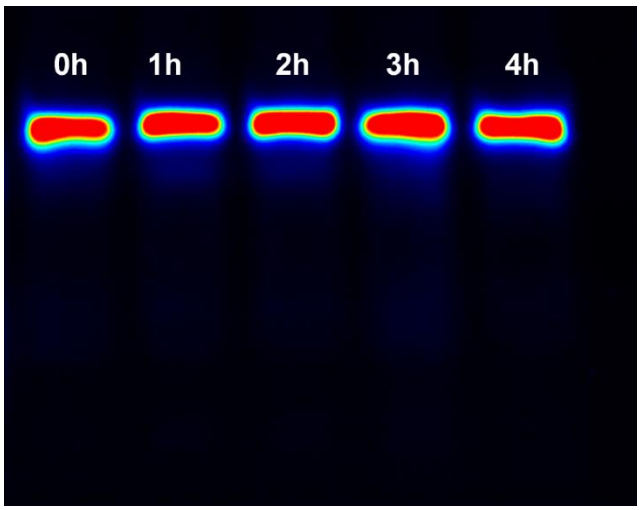


Fig. S4. Polyacrylamide gel electrophoresis analysis of DNA (1.0 μM) cleavage incubated by large CeO_2 particles (165 nm, 100 μg/mL) at 60°C for 4 h. The initial concentration of DNA (FAM-A_{15}) was 1.0 μM.

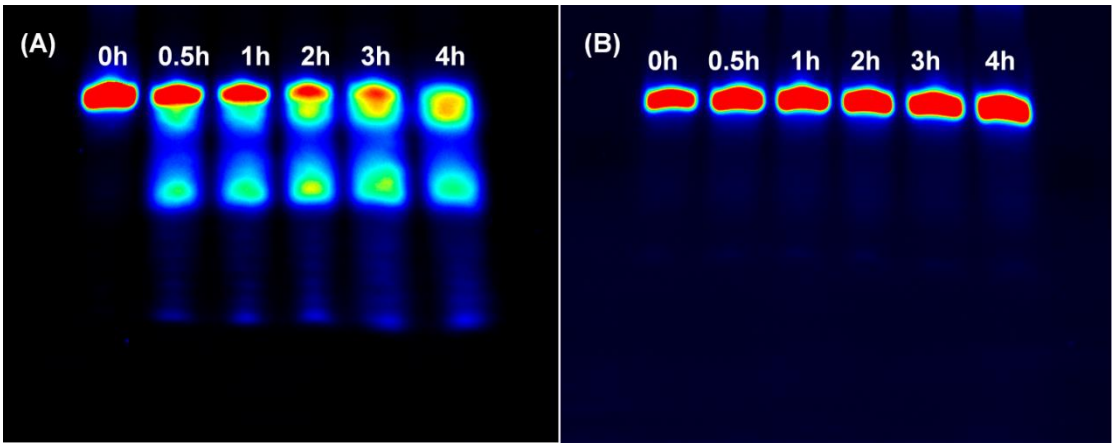


Fig. S5. Gel micrographs showing cleavage of DNA (FAM-A_{15}) by heat treated (A) 100 µg/mL CeO_2 at 60°C, and (B) DNase I (100 U/mL) at 37°C for 4 h. Before the test, both the CeO_2 and DNase I solutions were heated at 90ºC for 10 min. The initial concentration of DNA (FAM-A_{15}) was 1.0 µM. The DNase I sample showed no cleavage indicating that it was deactivated by the heat treatment.

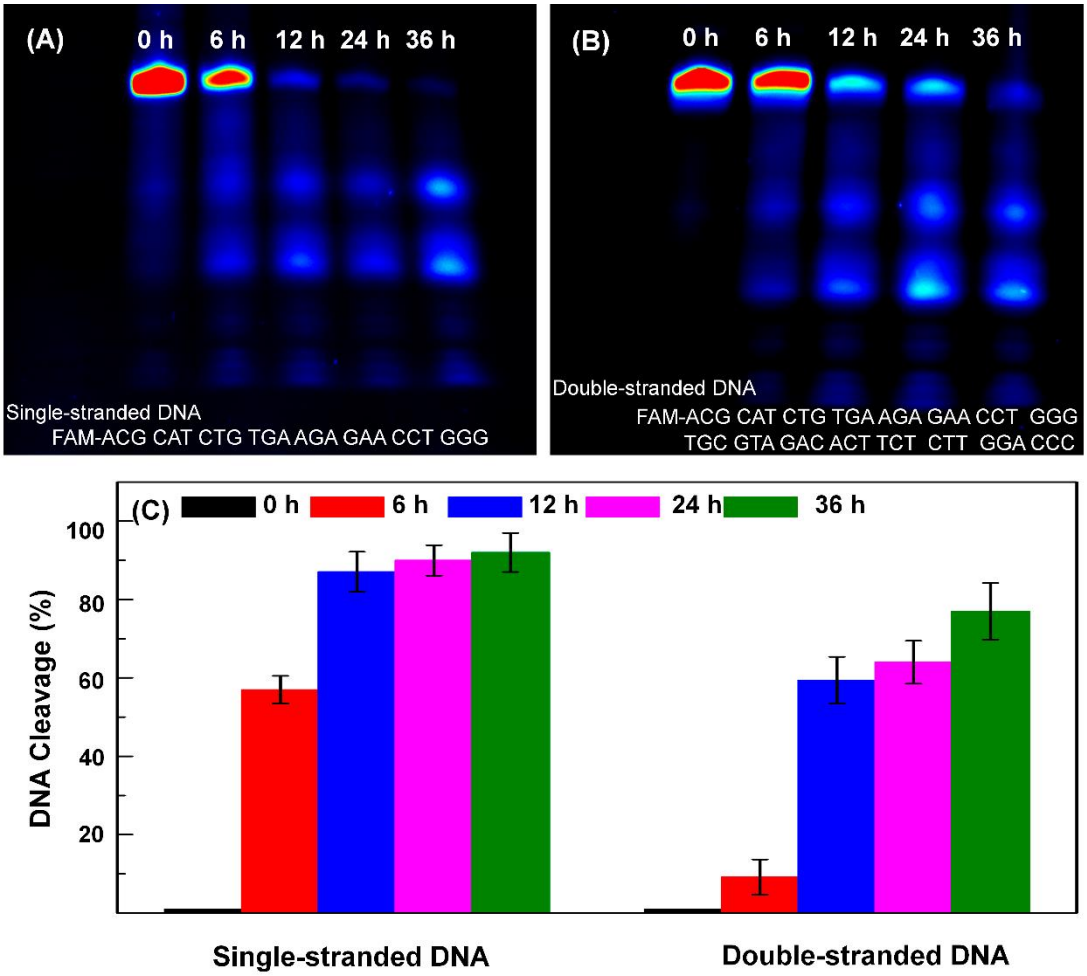


Fig. S6. Gel micrographs showing cleavage of a random sequenced 24-mer single-stranded DNA (A) and its duplex (B) by CeO_2 (100 μg/mL) at 37°C. The initial concentrations of single-stranded DNA and double stranded DNA were 1.0 μM. (C) Quantification of DNA cleavage yields of the single-stranded DNA (left) and double-stranded DNA (right) at different incubation times.

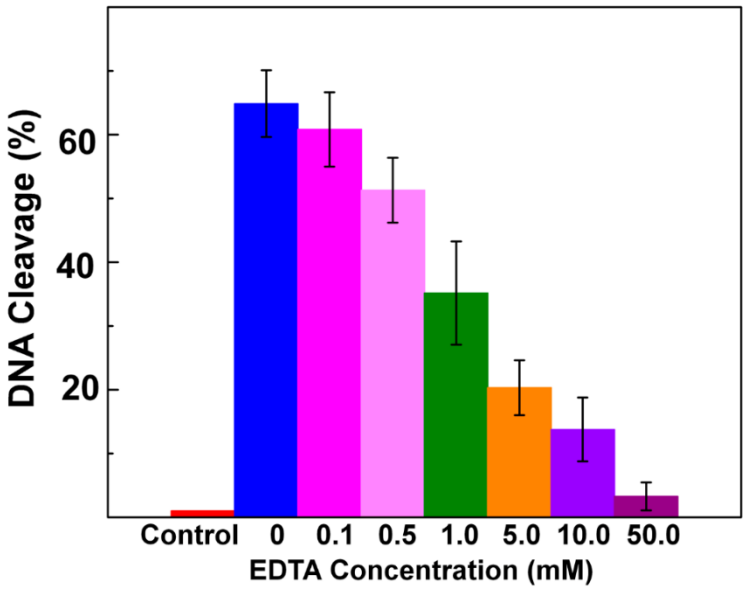
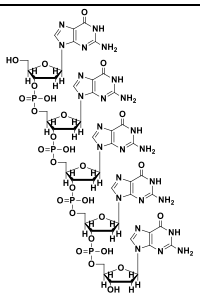
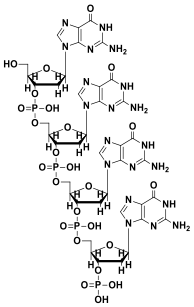
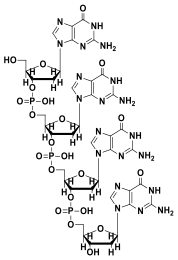
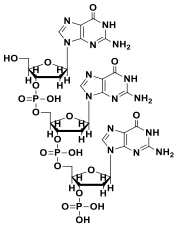
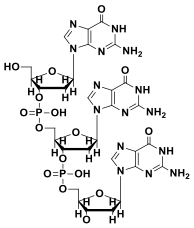
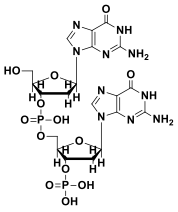


Fig. S7. Effect of EDTA (0-50 mM) on the FAM-A_{15} DNA cleavage yield by nanoceria at 60°C for 2 h. The initial concentration of the DNA was 1.0 μM. All experiments were performed in triplicate.

Table S1. The sequences of DNA used in this work

DNA	Sequences (from 5'to 3') and modifications
FAM-A_5	FAM-AAAAA
FAM-G_5	FAM-GGGGG
FAM-C_5	FAM-CCCCC
FAM-T_5	FAM-TTTTT
FAM-A_{15}	FAM-AAAAAAAAAAAAAAA
FAM-G_{15}	FAM-GGGGGGGGGGGGGGG
FAM-C_{15}	FAM-CCCCCCCCCCCCCCC
FAM-T_{15}	FAM-TTTTTTTTTTTTTTT
FAM-A_{20}	FAM-AAAAAAAAAAAAAAAAAAAA
FAM-A_{30}	FAM-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
FAM-A_{45}	FAM-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
FAM-A_{72}	FAM-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
	AAAAAAAAAAAAAAAAAAAAAAAAA
FAM-A_{90}	FAM-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
	AAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAA

Table S2. Main DNA cleavage products of G_5

No.	Possible cleavage products of DNA-G_5		MS analysis
	Chemical molecular formula	Theoretical mass	Experimental results
1		[M-H$^+$]=1582.29956 [M-2H$^+$]/2=790.64614 [M-3H$^+$]/3=526.76166	[M-2H$^+$]/2=790.64655 [M-3H$^+$]/3=526.76094
2		[M-H$^+$]=1333.21337 [M-2H$^+$]/2=666.10304 [M-3H$^+$]/3=443.732937	**Not detected**
3		[M-H$^+$]=1253.24704 [M-2H$^+$]/2=626.11988 [M-3H$^+$]/3=417.07749	[M-2H$^+$]/2=626.11970
4		[M-H$^+$]=1004.16085 [M-2H$^+$]/2=501.57678	**Not detected**
5		[M-H$^+$] =924.19452 [M-2H$^+$]/2=461.59362	[M-H$^+$]=924.19459 [M-2H$^+$]/2=461.59359
6		[M-H$^+$] =675.10883 [M-2H$^+$]/2 =337.05052	**Not detected**

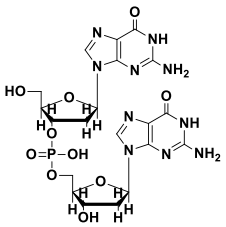
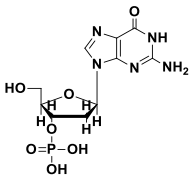
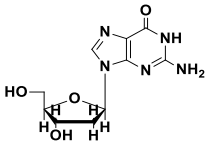
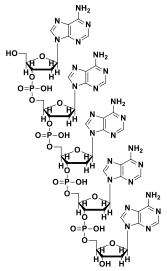
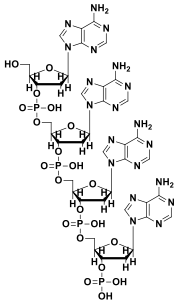
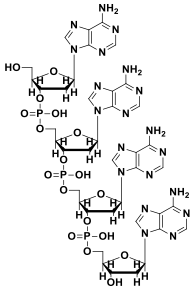
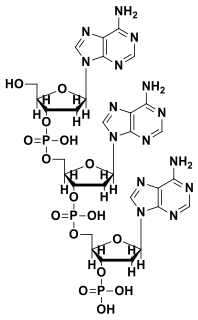
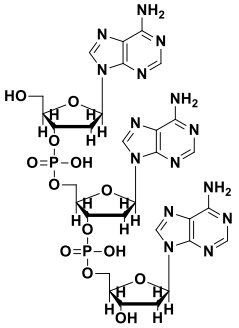
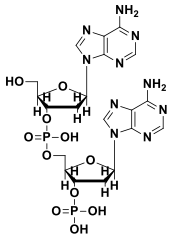
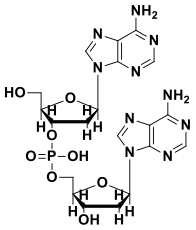
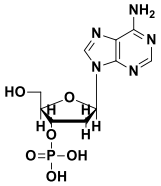
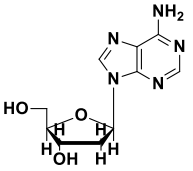
7		[M-H$^+$] =595.14200	[M-H$^+$]=595.14189
8		[M-H$^+$]=346.05581	**Not detected**
9		[M-H$^+$]=266.09675	[M-H$^+$]=266.21305

Table S3. Main DNA cleavage products of A_5

No.	Possible cleavage products of DNA-A_5		MS analysis
	Chemical molecular formula	Theoretical exact mass	Experimental results
1		[M-H$^+$]=1502.32498 [M-2H$^+$]/2=750.65885 [M-3H$^+$]/3=500.10347	[M-2H$^+$]/2=750.65886
2		[M-H$^+$]=1269.23371 [M-2H$^+$]/2=634.11321	**Not detected**
3		[M-H$^+$]=1189.26738 [M-2H$^+$]/2=594.13005	[M-2H$^+$]/2=594.12949
4		[M-H$^+$]=956.17610 [M-2H$^+$]/2=477.58441	**Not detected**
5		[M-H$^+$]=876.20977 [M-2H$^+$]/2=437.60125	[M-2H$^+$]/2=437.13792

6		[M-H$^+$]=643.11850 [M-2H$^+$]/2=321.05506	**Not detected**
7		[M-H$^+$]=563.15217	[M-H$^+$]=563.15177
8		[M-H$^+$]=330.06089	**Not detected**
9		[M-H$^+$]=250.09456	[M-H$^+$]=250.09482

References

1. B. Liu and J. Liu, *ACS Appl. Mater. Interfaces*, 2015, **7**, 24833-24838.
2. B. Liu, L. Ma, Z. Huang, H. Hu, P. Wu and J. Liu, *Mater. Horizons*, 2018, **5**, 65-69.
3. B. Liu, Z. Sun, P.-J. J. Huang and J. Liu, *J. Am. Chem. Soc.*, 2015, **137**, 1290-1295.
4. Y. Zhao, Y. Wang, A. Mathur, Y. Wang, V. Maheshwari, H. Su and J. Liu, *Nanoscale*, 2019.