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Cleaving DNA by nanozymes

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Abstract

DNA cleavage plays a crucial role in many biological processes such as DNA replication, transcription, and recombination. It is also a powerful tool in gene editing, therapeutics and biosensor design. Nanozymes aim to develop nanomaterial-based enzyme mimics. Compared with natural enzymes, nanozymes offer advantages of higher stability, lower cost, and recyclability. Recently, nanozymes with interesting DNA cleavage activities have emerged, including both hydrolytic and oxidative cleavage. This Perspective starts by introducing DNA cleavage of nanozymes, focusing on recent examples. Some interesting nanozymes include CeO_2 nanoparticles for the hydrolytic cleavage of single-stranded DNA oligonucleotides, chiral carbon dots mimicking topoisomerase activity, and light-assisted cleavage of DNA. The corresponding cleavage mechanisms are then discussed along with a few representative applications for DNA repair and as antibacterial agents. Finally, a few future research opportunities are discussed.

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1. DNA cleavage

As the carrier of genetic information, DNA is a highly stable molecule with an estimated half-life of 200 million years at ambient conditions.[1] DNA is linked by phosphodiester bonds with the inert and negatively charged phosphate backbone exposed, while the DNA bases are hidden in the helical structure. DNA cleavage is essential in biology. For example, restriction endonucleases recognize specific DNA sequences[2-4] and cleave foreign DNA to defend against viral infections.[5] Topoisomerases resolve topological problems during replication, transcription, and recombination by cleaving one or both strands of DNA.[6, 7] DNA cleavage, especially sequence-specific cleavage, is also critical in applications such as gene editing,[8-10] therapeutics,[11, 12] and biosensor design.[13, 14]

DNA cleavage can follow hydrolytic or oxidative mechanisms.[15] Most natural DNases perform hydrolytic cleavage, where the cleaved DNA fragments can be ligated to form a longer piece in the presence of a ligase. A typical mechanism of hydrolytic DNA cleavage is the nucleophilic attack by H_2O or OH^- at a phosphate, which is followed by the cleavage of phosphodiester linkages at the 3'-PO and the departure of the alcohol group (Figure 1A).[15] Since these nucleophiles originate from external molecules, they are far less efficient than the internal 2′-OH nucleophile for RNA cleavage. Metal ions often act as a Lewis acid to stabilize the additional negative charge generated in the intermediate.[15, 16] Oxidative DNA cleavage such as induced by Fe·EDTA/H_2O_2 occurs due to reactive oxygen species (ROS).[17, 18] Oxidative cleavage includes the modification of the deoxyribose (e.g. the hydrogen abstraction at C-3′ of deoxyribose sugar, Figure 1B),[15] and all four nucleobases (e.g. the addition of a hydroxyl radical to the C5 or C6 double bond of pyrimidines, and the C8 of purines, Figure 1C).[19]

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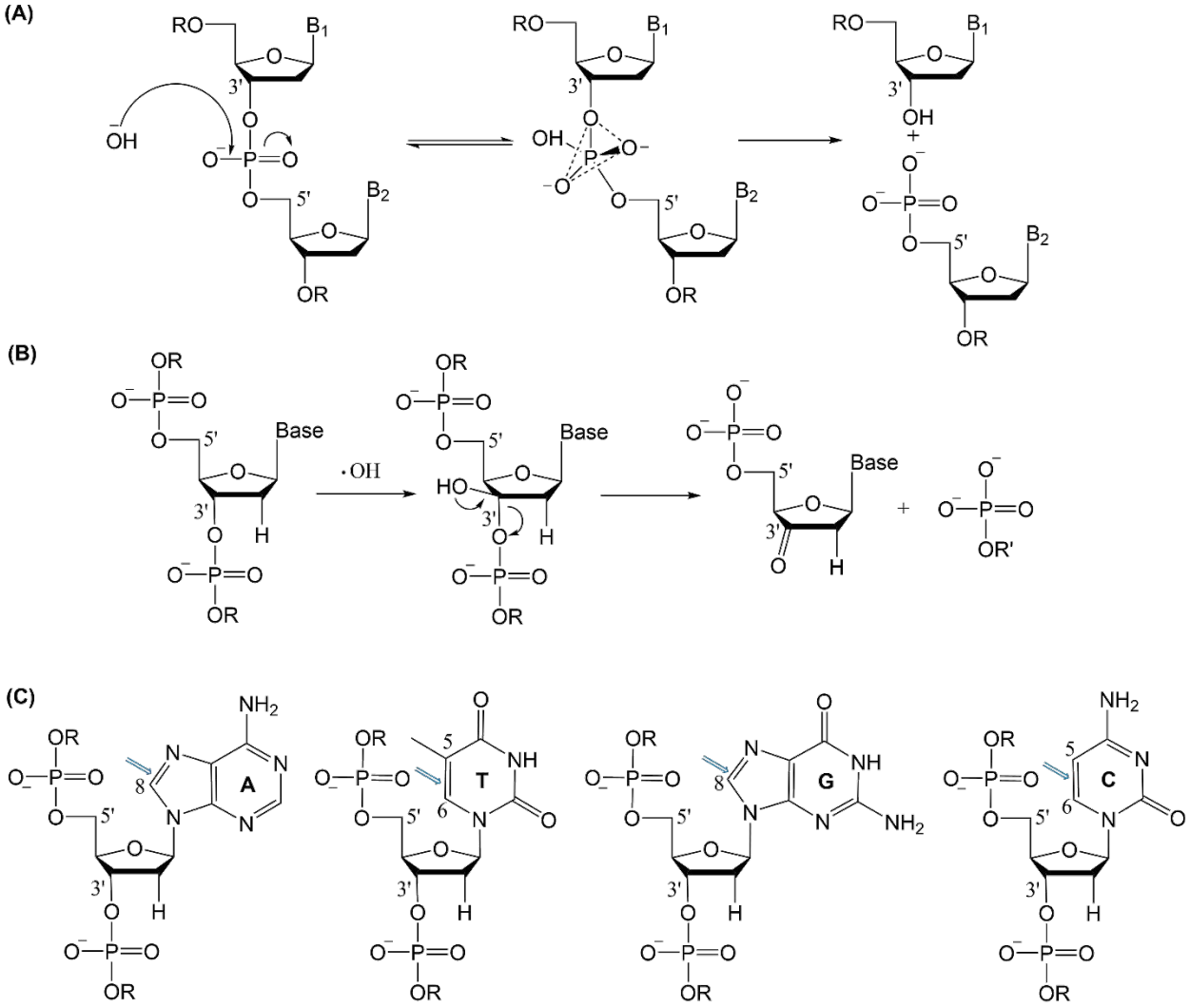

Figure 1. The types of DNA cleavage reactions. (A) Hydrolytic cleavage mechanism redrawn from ref.[15] Copyright 2014 Science and Education Publishing. (B) Oxidative cleavage reaction of hydrogen abstraction at C-3′ of the deoxyribose. Redrawn from ref.[15] Copyright 2014 Science and Education Publishing. (C) Oxidative addition sites of hydroxyl radicals to the DNA bases pointed by the blue arrows. Redrawn from ref.[19] Copyright 1993 Nature Publishing Group.

2. Nanozymes

With the discovery of the peroxidase-like activity of iron oxide nanoparticles in 2007,[20] the concept

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of nanozymes has taken off for its low cost, high stability, and interesting surface chemistry.[21-24] What distinguishes nanozymes from inorganic catalysts is the catalysis of biologically relevant reactions at close to physiological conditions.[25-28] Thus far, most nanozymes used small molecular substrates.[21, 22] A few nanozymes using DNA as substrates have also emerged, mimicking the activities of phosphatase,[29] photolyase,[30] nuclease,[31] and topoisomerase I.[32] There are a few types of nanozyme materials: gold-based,[33, 34] cerium-based,[31, 32, 35-38] carbon-based,[39-41] and quantum dots (QDs),[32, 38] with some showing restriction endonuclease or topoisomerase activity that cleaves DNA with precise fragment lengths, sites, and specificity (Table 1). In this article, we discuss nanozymes using its native surface instead of relying on the grafted ligands with nuclease activities.[33, 34, 42]

Table 1. Some DNA cleavage nanozymes, their substrates, reaction conditions, activities, and cleavage mechanisms.

Materials	**Substrates**	**Light**	**DNase**	**Mechanism**	**Ref**
CeO_2 nanoparticles	poly-A (single-stranded)	no	DNase I	hydrolytic cleavage	[35]
cys-CdTe QD	duplex DNA over 90 bp	circularly polarized light	restriction endonuclease (at GAT'ATC)	hydrolytic cleavage (ROS ·OH)	[38]
Ce-based Fe_3O_4/SiO_2 core/shell	extracellular DNA	no	DNase I	hydrolytic cleavage	[36]
Ce-based /MOF	extracellular DNA	no	DNase	hydrolytic cleavage	[37]

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graphene oxide/Cu^{2+}	supercoiled PSICOR-GFP DNA	no	undefined	oxidative and hydrolytic cleavage	[39]
fullerene carboxylic acid	supercoiled pBR322 DNA	visible light	nuclease (at guanine)	oxidative cleavage (ROS singlet oxygen)	[40]
fullerene - oligonucleotide conjugates	single-stranded DNA, duplex DNA, hairpin DNA	light (λ> 310 nm)	nuclease (at guanine)	oxidative cleavage (ROS singlet oxygen)	[41]
graphene oxide	15-mer single-stranded DNA	UV or blue light	nuclease	oxidative cleavage (ROS $^1O_2$ and $O2^{\bullet-}$)	[31]
cysteine-derived carbon dots	supercoiled plasmid	no	topoisomerase I	oxidative cleavage (ROS ·OH)	[32]

3. Hydrolytic DNA Cleavage by Nanozymes

Our lab recently reported that CeO_2 nanoparticles of approximately 3-5 nm in size can cleave single-stranded DNA oligonucleotides.[35] DNA was adsorbed on CeO_2 in many previous experiments[43-45] for the detection of various analytes,[46-49] but cleavage of DNA was not reported. We observed cleavage when the temperature was raised from the room temperature of around 22°C to the body temperature of 37°C. Different DNA homopolymers were all cleaved, although the patterns of product distribution were different. At 60°C, the cleavage of G_{15} DNA (15-mer polyguanine) reached a rate constant of 1.68 h^{-1}, while the slowest A_{15} DNA reached 0.75 h^{-1}. Regardless of the DNA's initial length, the final products were mainly around 5-mer, which could be explained by the tighter adsorption of longer DNA. Once the DNA was cleaved down to around 5-mer, the affinity was too low to be stably adsorbed, and thus the cleavage stopped. This product

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distribution (Figure 2A) was quite similar to that of DNase I (Figure 2B). Mass spectrometry analysis found only hydrolytic cleavage products, and interestingly, all the terminal phosphate groups were dissociated. This could be explained by the phosphatase-like activity of CeO_2.[50] Ce(IV) is known to have DNA cleavage activity attributable to dinuclear species.[51] Since the surface of CeO_2 may contain similar oxygen-bridged active centers, the cleavage by CeO_2 can be explained (Figure 2C). Interestingly, other tested metal oxide nanoparticles failed to show such a DNA cleavage activity.

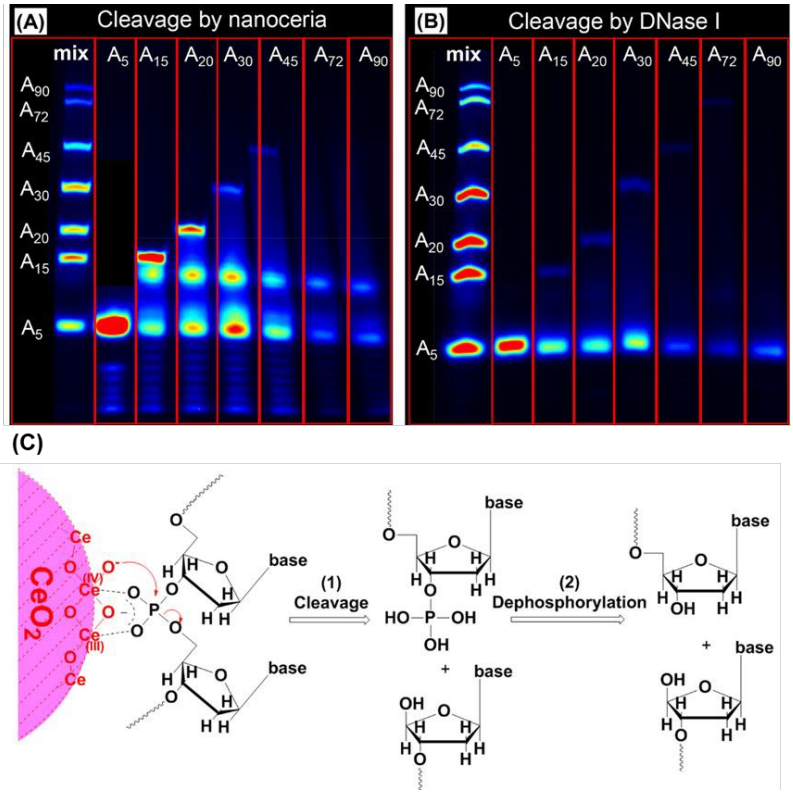

Figure 2. Gel micrographs showing the cleavage of poly-A DNA of various lengths by (A) 100 μg mL^{-1} CeO_2 at 60°C, and (B) DNase I (100 U mL^{-1}) at 37°C for 1 h. The leftmost lanes are a mixture of the untreated DNAs. (C) A proposed DNA cleavage mechanism on CeO_2: cleavage followed by dephosphorylation. Reprinted with permission from ref.[35] Copyright 2019 the Royal Society of Chemistry.

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4. Oxidative DNA Cleavage by Nanozymes

Oxidative DNA cleavage by nanozymes is more commonly reported. Some early examples include fullerene derivatives,[40, 41] and graphene oxide (GO) in the presence of Cu^{2+} ions.[39] Zhang and coworkers reported that after incubating the PSICOR-GFP DNA (a plasmid DNA) with Cu^{2+} and a GO sheet,[39] the supercoiled DNA converted to the relaxed form and the corresponding bands were separated by agarose gel electrophoresis. Fluorescence spectroscopy suggested that the planar GO sheets intercalated into the DNA. The type of cleavage in the GO/Cu^{2+} system depended on the location of Cu^{2+} ions on DNA. When Cu^{2+} was on the sugar phosphate backbone, the Lewis acid property of Cu^{2+} could promote hydrolytic DNA cleavage. Whereas when Cu^{2+} was bound to the electron-donating nucleobases, oxidative DNA strand cleavage could be indirectly promoted. Another example of oxidative cleavage was induced by the chiral cysteine-derived carbon dots (CDs) recently reported by Yang and coworkers.[32] After incubating with CDs at 37°C for 3 to 24 h, a supercoiled plasmid DNA gradually transformed into a nicked open-circular configuration by creating single-stranded breaks in the double helix. The inhibition of DNA cleavage by replacing oxygen with saturated N_2 in the DNA suggested an oxidative cleavage mechanism. Interestingly, both L and D-cysteine were used to prepare the CDs, but the D-CDs intercalated with the DNA more strongly and showed stronger cleavage activity than the L-CDs (Figure 3A). The authors proposed that the CDs were able to produce hydroxyl radicals as the active ROS for DNA cleavage.

5. Light-dependent DNA cleavage

Light plays a critical role in DNA damage, the most common example of this is the formation of thymine dimers.[52] Recently a CeO_2-based nanozyme was reported to mimic the photolyase activity and break thymine dimers.[30] Light-dependent nanozymes have been extensively explored,

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including the cleavage of DNA.[53] The role of light can be divided into two major types. In type I, superoxide radicals are generated from molecular oxygen via electron transfer, while in type II singlet oxygen is generated using photosensitizers.[15] With the help of light, our lab reported a metal-free GO system (no Cu^{2+}).[31, 53] DNA cleavage occurred only when both GO and light were present, with shorter wavelength light further promoting the cleavage yield. Using single-stranded FAM-labeled C_{15}, A_{15}, G_{15} and T_{15} DNA as substrates, cleavage product bands were observed by gel electrophoresis in the presence of GO and 365 nm light. Since guanine is most easily oxidized among the nucleobases,[54] the highest cleavage rate of FAM-G_{15} (Figure 3B) hinted oxidative cleavage.

Kuang and coworkers reported that chiral cysteine-modified tetrahedral CdTe QDs can mimic a restriction endonuclease under photo-excitation by circularly polarized light (Figure 3C).[38] Agarose gel electrophoresis indicated that double-stranded DNA of over 90 base pairs can be gradually separated into two distinct fragments at particular thymine and adenine sites after 2 h of exposure to the L-cys CdTe and D-cys CdTe. Light-induced ROS was shown to be responsible for the cleavage, and it yielded a cleavage product at the GAT'ATC restriction site, just like the restriction endonuclease *EcoRV*. [55] This work also showed that the photo-induced DNA cleavage performed well in human cervical cancer cells, rat fetal neural stem cells, and HeLa tumor bearing nude mice.

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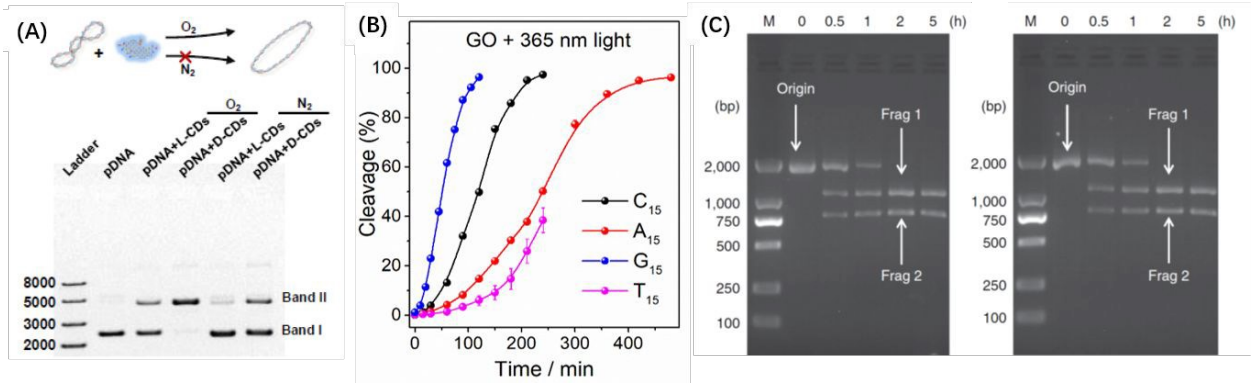

Figure 3. (A) Gel electrophoresis analysis of supercoiled plasmid DNA treated with the L-CDs and D-CDs for 24 h in an aerobic environment and in N_2, and a cartoon showing the need of oxygen. Reprinted with permission from ref.[32] copyright 2020 Wiley-VCH. (B) Cleavage kinetics of the four types of DNA homopolymers by the light-driven GO. The cleavage yields were quantified by the ratio between the product band and the total band intensity. Reprinted with permission from ref.[31] copyright 2020 Tsinghua University Press and Springer. (C) Electrophoresis micrographs of the L-Cys CdTe (left) and D-Cys CdTe (right) nanoparticles with a 1,839 bp DNA illuminated with 405 nm left- or right-handed circularly polarized light for 2 h. The two DNA products after cleavage are denoted Frag 1 and Frag 2, respectively. Reprinted with permission from ref.[38] Copyright 2018 Springer Nature.

6. DNA cleavage process and mechanism

In general, most nanozymes follow the process of adsorption, surface reaction, and product desorption, similar to typical heterogeneous catalysts.[23] This process was also used to describe DNA cleavage. DNA can adsorb on many nanomaterials via various interactions. For example, CeO_2 can absorb with the phosphate backbone of DNA due to the cerium ions,[35, 56, 57] while GO may insert into the base pairs of DNA double helix, or adsorb single-stranded DNA via π-π

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stacking and hydrogen bonding.[53, 58, 59] Following DNA adsorption, cleavage reactions turn DNA into fragments until the product length becomes short enough to desorb from the nanozymes due to a weakened adsorption affinity. After desorption, the surface sites are regenerated, allowing for the adsorption of new substrates in the next catalytic cycle.[23] Two such examples are shown in Figure 4. From the above discussion, the adsorption of DNA is very important. For example, DNA cleavage by CeO_2 nanoparticles was inhibited by adding inorganic phosphate ions or EDTA to cap the CeO_2 surface.[35] In addition, molecular dynamics (MD) simulation can also reveal the interactions between nanozymes and DNA.[32]

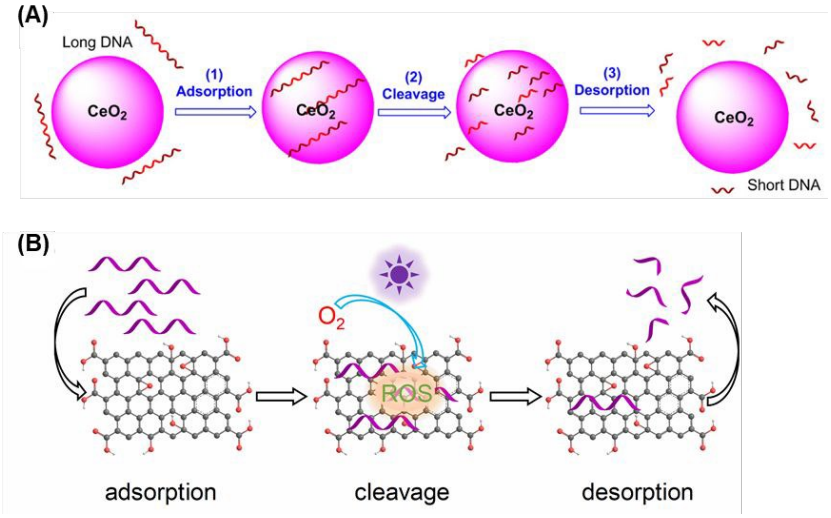

Figure 4. (A) Schematic representation of DNA cleavage by CeO_2, including adsorption, cleavage, and desorption once the substrate became sufficiently short. Reprinted with permission from ref.[35], copyright 2019 The Royal Society of Chemistry. (B) Schematic diagram of GO as a photocatalytic nanozyme for cleaving single-stranded DNA. The cleavage stops when the product becomes sufficiently short and then desorbs from GO. Reprinted with permission from ref.[31], copyright 2020 Tsinghua University Press and Springer.

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To characterize DNA cleavage, gel electrophoresis is a simple yet powerful tool. Kinetic information can be extracted to provide mechanistic insights. For example, for the GO-based DNA cleavage, the sigmoidal shape kinetics suggested an accumulation of ROS, while the faster cleavage of FAM-G_{15} supported an oxidative cleavage mechanism (Figure 3B).[31] In addition, T4 DNA ligase can serve as evidence of hydrolytic DNA cleavage, though some oxidative cleavage reactions still yielded hydrolytic products.[32, 60]

To further clarify DNA cleavage mechanisms, cleavage products can be characterized by mass spectrometry, and the topological rearrangements of DNA can be analyzed by agarose gel electrophoresis (Figure 3A) and atomic force microscopy (AFM).[32, 35] Moreover, important ROS can be identified by adding corresponding scavengers.[53] Typically, superoxide dismutase (SOD), tryptophan or DMSO, mannite, and catalase are used as scavengers of $O_2^{\cdot-}$, $^1O_2$, ·OH, and H_2O_2, respectively. Electron spin resonance (ESR) spectroscopy is useful for further confirming the production of ROS. For example, after adding trapping agents 5,5-dimethyl-1-pyrroline-noxide (DMPO) and 2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPO), the ESR signals of the DMPO/·OH and TEMPO/$^1O_2$ adducts can be respectively detected.[31, 32]

7. Comparison of nanozymes and protein-based nucleases

Given the diverse nature of the reviewed nanozymes, it is hard to have a general comparison with nucleases. Nevertheless, the CeO_2 example in Figure 2 provides an interesting comparison between a particular nanozyme and a nuclease, DNase I.[61] Their differences are mainly in the following points. (1) Although the main products generated by both were both around 5-mer, the CeO_2 nanoparticles can cleave DNA into even smaller products shorter than 5-mer. In addition, by counting the bands below the 5-mer marker, there are more than 5 bands (Figure 2A), suggesting sub-nucleotide resolution likely due to further dephosphorylation. (2) DNase I is 100~500 fold

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more active in cleaving double-strand DNA than single-stranded DNA,[62] whereas the CeO_2 nanoparticles showed comparable cleavage for them. (3) Cleavage rate: 100 $\mu g \cdot mL^{-1}$ CeO_2 was about 100-fold less active than 100 $U \cdot mL^{-1}$ DNase I in cleaving FAM-A15 at 37 °C, although the weight concentration of the DNase I was less than 1% of the CeO_2. (4) The CeO_2 nanoparticles exhibit higher stability and lower price than DNase I. DNase I can be irreversibly denatured and inactivated at above 67°C,[63] whereas the CeO_2 nanoparticles remained high activity even at 90°C. In fact, the higher the temperature, the higher the activity for CeO_2. Overall, although the activity of nanozymes are not yet comparable to that of natural nucleases, they have a few advantages especially for cost and stability considerations.

8. Applications of nanozyme-mediated DNA cleavage

DNA-cleaving nanozymes have many interesting applications, such as gene editing, DNA repair, and antibacterial agents, which may help in the treatment of skin diseases, bacterial infection and potentially biosensing. Gene editing relies on nucleases for gene manipulation.[8] Yang and coworkers found that their D-CDs can transform the topological configuration of supercoiled DNA into the nicked open-circular configuration.[32] To verify whether the CDs could promote transcriptional elongation like topoisomerase I, the p-eGFP plasmids were treated with the CDs and their expression of green fluorescent protein (GFP) in a cell-free protein synthesis (CFPS) system was evaluated. Interestingly, the CDs enhanced the expression of GFP in comparison to the untreated plasmids, attributable to the nicked sites facilitating the supercoiling relaxation during transcription.

DNA repair is an important process that maintains the integrity of the genome. Several studies focused on DNA repair properties of nanomaterials.[64] Tian et al. reported porous cerium nanorods (PN-CeO_2) with DNA photolyase activity for repairing UV-induced DNA damage and

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selectively triggering cyclobutane pyrimidine dimers into thymine monomers.[30] Intracellular and *in vivo* experiments showed that the PN-CeO_2 effectively protected the HaCaT cells from oxidative damage by reducing UV-induced ROS levels.

The antibacterial applications of nanozymes were summarized in some excellent reviews.[22, 25] Nanozymes with DNA cleavage activities are potential antimicrobial candidates. Qu and coworkers developed a DNase-mimetic nanozyme for combating bacterial biofilms.[36] The nanozyme was obtained by conjugating gold nanoparticles with Ce(IV) nitrilotriacetic acid complexes on Fe_3O_4/SiO_2 core/shell particle scaffolds. Since extracellular DNA (eDNA) is a necessary component in the formation of biofilms, the cleavage of eDNA can be used for anti-biofilm applications. After attaching S. aureus cells to a nanozyme-coated surface, the biofilm became thinner. The amount of eDNA indeed decreased, as revealed by quantitative real-time PCR analysis. Notably, the nanozyme appeared more stable and recovered more readily than DNase, and it also enhanced the ability of classic antibiotics to kill biofilm-based bacteria.

To avoid bacterial recolonization and biofilm regrowth, Qu and coworkers further developed a series of nanozymes with DNase and peroxidase dual activities containing the cerium (IV) complexes to hydrolyze eDNA, and a metal-organic framework (MOF) with peroxidase-like activity can help kill bacteria.[37] Confocal laser scanning microscopy and scanning electron microscopy (SEM) images showed that this dual nanozyme formulaiton disintegrated the biofilms and inhibited bacterial growth, while *in vivo* tests also exhibited good wound healing and antibacterial effects.

9. Conclusions and future perspectives

In this Perspective, recent developments in DNA-cleaving nanozymes are summarized, including the two types of DNA cleavage reactions, various nanozyme materials, mechanisms, and a few

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applications. Both hydrolytic and oxidative cleavage have been reported, although the latter is more common. Light provides an additional energy source to boost DNA cleavage in many systems. Despite the progress, further work is needed to clarify the DNA cleavage mechanism and promote the cleavage activities of nanozymes. First, the kind of nanozymes with DNA cleaving activities needs to be expanded. Various nanoparticles can be screened for DNA cleavage activity. Since cerium appears to be an important metal found in many formulations, cerium doped materials can be synthesized. Second, factors influencing cleavage efficiency and specificity need to be understood to help improve the performance of DNA cleavage nanozymes compared with natural enzymes. Not only does light provide energy, but it also renders controlled cleavage with a high spatial and temporal resolution.[53, 65] So far, most of the cleavage reactions required UV or blue light, which has a short tissue penetration depth. Using red and near IR light can solve this problem, while the involvement of upconversion materials might be useful.[66-68] To further increase specificity of DNA cleavage, learning from nature to use a DNA or DNA oligonucleotide to guide the cleavage site for nanozymes is also an interesting future direction, and this has been nicely demonstrated in the CRISPR system. Finally, it would be useful to go from cleaving DNA in buffers to more practical examples of cleaving viral, bacterial, and cellular DNA, especially considering nanomaterials can stick, disrupt, and enter cells.[69-71] It is difficult to predict the effect of biological sample matrix on the activity of different nanozymes and this has to be tested individually. In particular, the surface properties of nanozymes in biological matrices need to be elucidated to ensure that high activity can be retained.

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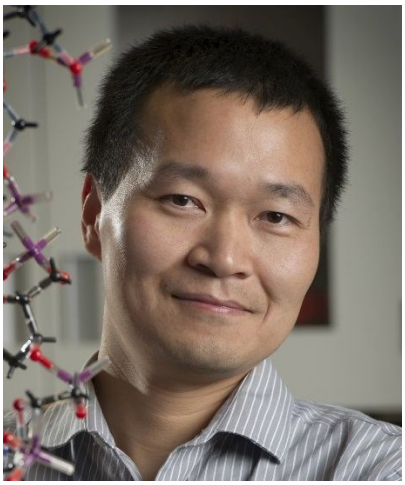

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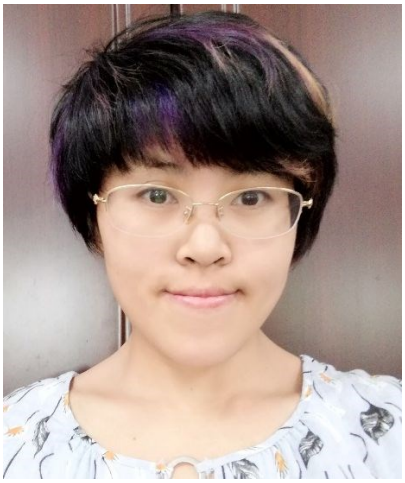

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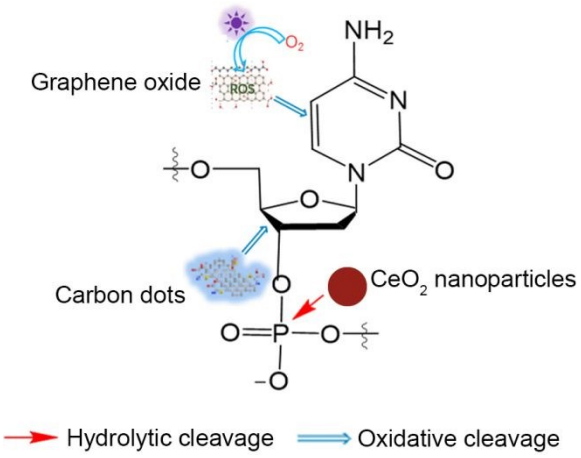

Various nanomaterials can mimic the activities of nucleases for hydrolytic and oxidative DNA cleavage on different sites allowing interesting biomedical and bioanalytical applications.

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