

## Design of nanozymes for inflammatory bowel disease therapy

Chaoqun Cheng<sup>1,2</sup>, Sheng Zhao<sup>1,2</sup>, Yuan Cheng<sup>1,2</sup>, Yufeng Liu<sup>1,2</sup> & Hui Wei<sup>1,2\*</sup>

<sup>1</sup>Department of Biomedical Engineering, College of Engineering and Applied Sciences, Nanjing National Laboratory of Microstructures, Jiangsu Key Laboratory of Artificial Functional Materials, Nanjing University, Nanjing 210023, China;

<sup>2</sup>State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Chemistry and Biomedicine Innovation Center (ChemBIC), Nanjing University, Nanjing 210023, China

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Nanozymes, the functional nanomaterials with enzyme-like activities, have been widely explored for both *in vitro* and *in vivo* applications (Wei and Wang, 2013; Wu et al., 2019; Huang et al., 2019; Liang and Yan, 2019). Particularly, by modulating the levels of reactive oxygen species (ROS) *in vivo*, numerous therapeutic nanozymes have been developed to treat diseases, ranging from cancer to neurodegenerative diseases (Fan et al., 2018; Kwon et al., 2018). Despite these successes, applying the therapeutic nanozymes to clinical translation is still full of difficulties and challenges. We performed an analysis on the *in vivo* applications of nanozymes to identify these challenges (Figure S1 in Supporting Information). It revealed that first, both pristine and functionalized nanozymes are used for *in vivo* applications, and functionalized nanozymes are dominant (60.08%). Among the functionalized nanozymes, only 11.02% have the targeting moieties. It indicates that 88.98% of nanozymes are not able to specifically target disease sites, which would in turn lead to severe adverse effects. Second, only 3.8% of the *in vivo* nanozymes can be cleared or degraded and then eliminated. Therefore, the clearable or degradable nanozymes are needed to minimize their long-term toxicity concerns.

Recently, growing efforts have been devoted to gradually tackling the above mentioned challenges. Herein, we take inflammatory bowel disease (IBD) therapy as an example to

highlight such efforts and to propose a possible approach to translational nanozymes. IBD is used to describe disorders that involve chronic inflammation of digestive tract, which can lead to several complications such as severe dehydration, ulcers, fistulas, dementia, and even colon cancer (Pardi et al., 1998). Generally, IBD includes two main forms: ulcerative colitis (UC) and Crohn's disease (CD). Various drugs for IBD are available, but their therapeutic outcome is not satisfactory, which is partially due to the lack of specificity of these treatments (Table S1 in Supporting Information). Therefore, it is urgent to develop more effective treatments for IBD.

IBD has several distinct pathological characteristics, such as elevated levels of ROS and an accumulation of positively charged proteins at the disease site. These excessive ROS breaks the cellular redox homeostasis and consequently causes damages to cellular molecules such as lipids, proteins, and DNA. In addition, it is established that excessive ROS plays a critical role in both initiation and progression of IBD (Aviello and Knaus, 2017). Therefore, nanozymes with ROS scavenging abilities could act as effective therapeutics for IBD therapy. Meanwhile, one can also take advantage of the positively charged proteins at the disease site to design negatively charged drugs and drug carriers. Such drugs and drug carriers would provide an effective strategy for targeted therapy. In the following, taking the above two pathological characteristics into consideration, we discuss the recent progress in nanozyme-based IBD therapy.

\*Corresponding author (email: [weihui@nju.edu.cn](mailto:weihui@nju.edu.cn))

**Enhanced ROS scavenging activity of nanozymes for IBD therapy.** Both stoichiometric antioxidants and ROS scavenging enzymes can be used to eliminate the excessive ROS in IBD. However, these antioxidants and enzymes have their own limitations. The antioxidants are consumed as they eliminate ROS, therefore repeated dosing is usually needed. The enzymes are not very stable and many enzymes cause immunological responses. In contrast, nanozymes are not only more stable and cost-effective, they can also eliminate ROS catalytically, thus have great potential for the treatment of IBD.

We reported that the glutathione peroxidase (GPx)-like metal-organic framework (MOF) nanozyme could be used to treat colitis mice. The GPx-like activity of MIL-47(V)-X (MIL stands for Materials of Institute Lavoisier; X=F, Br, NH<sub>2</sub>, CH<sub>3</sub>, OH, and H) was modulated by a ligand engineering strategy. We found that MIL-47(V)-NH<sub>2</sub> had the highest GPx-like activity *in vitro*. Compared with the less active MIL-47(V)-H, MIL-47(V)-NH<sub>2</sub> showed an enhanced therapeutic efficacy for the treatment of colitis mice, demonstrating that systematic analyses of the structure-activity relationship are able to guide the design of high performance nanozymes for therapy (Figure 1A) (Wu et al., 2021).

In addition, as multiple ROS are involved in IBD, nanozymes with multiple ROS scavenging activities would be more effective for IBD therapy. For ROS scavenging activities of natural enzymes, natural superoxide dismutase (SOD) eliminates superoxide radical ( $\cdot\text{O}_2^-$ ), and natural catalase (CAT) and GPx eliminate hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), respectively. No natural enzyme can scavenge hydroxyl radical ( $\cdot\text{OH}$ ), an important and highly active ROS involved in IBD (Dandona et al., 1996). To this end, Prussian blue (PB) nanozyme with SOD and CAT-like activity as well as hydroxyl radical scavenging activity was prepared for IBD therapy. When the poly(vinylpyrrolidone)-modified PB nanozyme was intravenously administered, it reduced colitis in mice via scavenging ROS including  $\cdot\text{OH}$  and H<sub>2</sub>O<sub>2</sub> (Figure 1B) (Zhao et al., 2018).

While a single component nanozyme like PB could scavenge multiple ROS, there may be a competition among the different enzyme-like activities because of the shared active sites. By introducing multiple active sites within a single nanozyme, the multiple enzyme-like activities could be enhanced. We recently developed a rationally designed nanozyme to mimic the SOD/CAT cascade reactions for ROS elimination and IBD treatment. The designed Pt@PCN222-Mn nanozyme exhibited synergistic ROS-scavenging activity both *in vitro* and *in vivo*. More encouragingly, in both UC and CD mice models, the optimized Pt@PCN222-Mn nanozyme showed better therapeutic efficacy than 5-aminosalicylic acid (5-ASA), a medication for IBD treatment, under the same dosage (Figure 1C) (Liu et al., 2020).

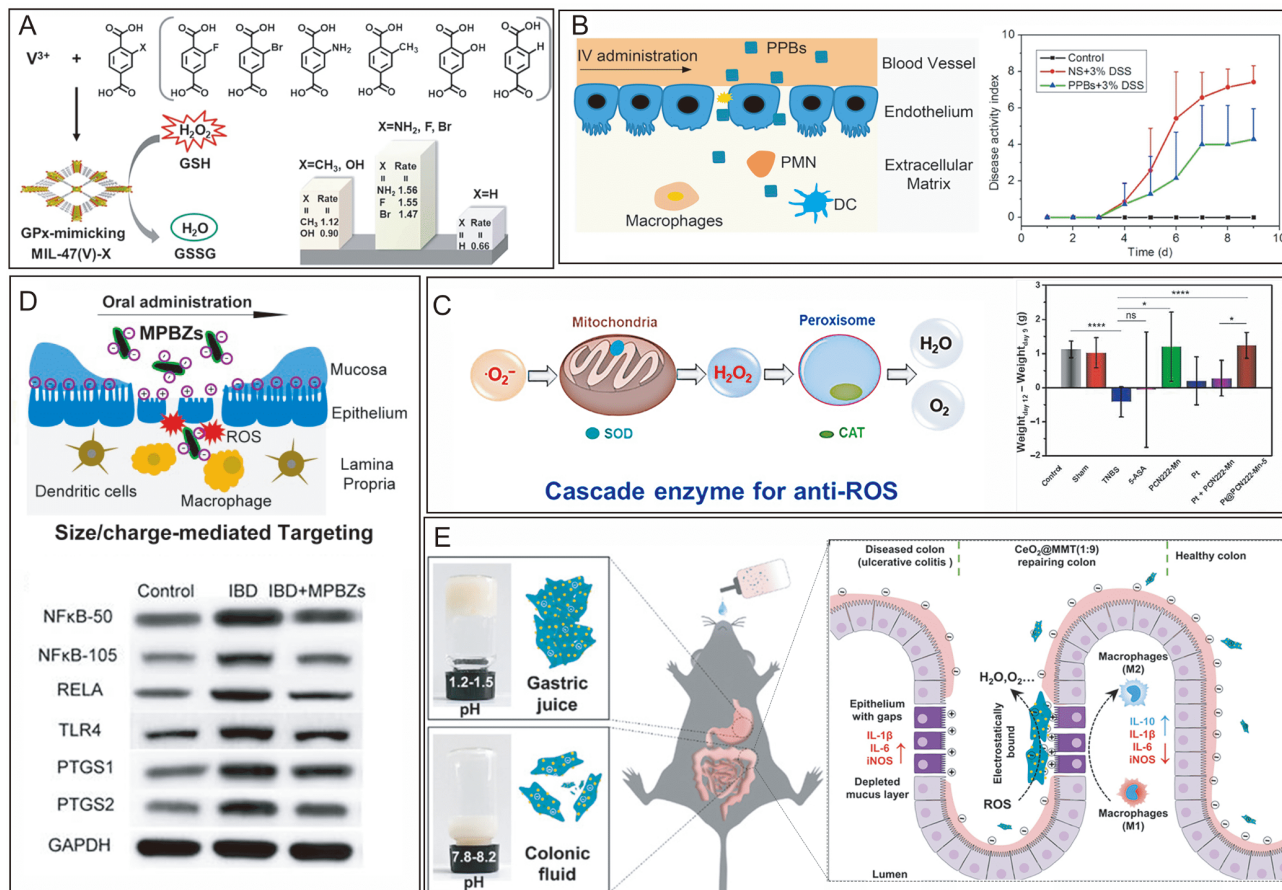
**Targeted ROS scavenging activity of nanozymes for**

**IBD therapy.** While the above studies have illustrated the promise of ROS scavenging nanozymes for IBD therapy, several concerns have not been fully addressed. First, whether the nanozymes target the whole colon or inflamed colonic lesions remains unknown. Second, nanozymes were administered intravenously or intraperitoneally in the past research. Such administration methods are not only inconvenient for patients, they may also lead to inevitable systemic absorption and associated toxicity concerns. A few recent reports have attempted to address these concerns.

As analyzed above, by using negatively charged drugs and drug carriers, one shall be able to target the positively charged disease site for better IBD therapy. Recently, a negatively charged PB nanozyme was prepared and orally administered for IBD therapy. The *ex vivo* study showed that the negatively charged PB nanozyme was able to target the inflamed colonic sites preferentially. Interestingly, such a preferential targeting was dependent on the size of PB nanozymes. The possible mechanism of the PB-based therapy was also studied, revealing a primary effect of PB nanozymes on the Toll-like receptor (TLR) signaling pathway (Figure 1D) (Zhao et al., 2019).

We have designed an orally administered CeO<sub>2</sub>@montmorillonite (CeO<sub>2</sub>@MMT) nanozyme to target the inflamed colon for IBD therapy. CeO<sub>2</sub> was chosen because of its SOD- and CAT-like activities as well as hydroxyl radical scavenging activity. MMT (a clinical drug) was chosen because it is a clinically used drug and negatively charged. By *in situ* growing CeO<sub>2</sub> nanoparticles (NPs) on sheets of MMT, the formed CeO<sub>2</sub>@MMT nanozyme was stable in simulated gastric fluids (pH 1.2–1.5) and negatively charged. When orally administered, CeO<sub>2</sub>@MMT nanozyme was efficiently delivered through the digestive tract, preferentially targeted the positively charged inflamed sites via electrostatic interaction, and alleviated the inflammation. In addition, CeO<sub>2</sub>@MMT minimized the systemic absorption of CeO<sub>2</sub> NPs and thus successfully avoided the toxicity (Figure 1E) (Zhao et al., 2020).

In summary, with the rapid development of nanozymes, more and more researches have been devoted to their potential translational applications, as the use of nanozymes for IBD therapy highlighted above. To advance the translational application, nanozymes could be combined with some drugs and/or devices already on the market. When nanozymes and clinical drugs are carefully paired, they may benefit each other mutually, as in the case of CeO<sub>2</sub> (a ROS scavenging nanozyme) growing on MMT. In addition, targeting the disease site would provide an effective strategy for IBD therapy, and enema also was an effective way for targeted therapy, such as an inflammation-targeting hydrogel for enema-based therapy in IBD (Zhang et al., 2015). Considering convenience and comfort, oral administration was the best choice in clinical administration. Nevertheless, to fully fulfill



**Figure 1** Nanozymes for inflammatory bowel disease therapy. A, MIL-47(V)-X ( $X=F, Br, NH_2, CH_3, OH,$  and  $H$ ) MOFs were successfully fabricated with rationally regulated GPx-mimicking activities by vanadium metal nodes, and MIL-47(V)- $NH_2$  showed the best performance to scavenge ROS (reprinted from Wu et al. (2021) with permission from John Wiley and Sons). B, Poly(vinylpyrrolidone)-modified Prussian blue (PPB) reduced colitis via scavenging ROS (reprinted from Zhao et al. (2018) with permission from American Chemical Materials). C, Cascade nanozymes for SOD and CAT catalyzed reactions possess a higher therapeutic efficacy than 5-ASA, by which IBD can be effectively treated (reprinted from Liu et al. (2020) with permission from American Association for the Advancement of Science). D, Size/charge-mediated MPBZs targeted positive surface in colitis mice and reduced colitis via TLR signaling pathway (reprinted from Liu et al. (2020) with permission from Ivyspring International). E,  $CeO_2@MMT$  nanozyme was stable at pH 1.2–1.5, passing through the digestive tract and targeted inflamed site via oral administration (reprinted from Zhao et al. (2020) with permission from John Wiley and Sons).

the translational promise of nanozymes for IBD therapy, there are still numerous challenges to be addressed. First, deep mechanistic studies are needed to understand the function of nanozymes towards IBD. For example, the roles of other important factors besides ROS should be systematically investigated (Figure S2 in Supporting Information), which include the role of gut epithelial cells in maintaining mucosal homeostasis, the augmented ROS production by mucosal immune cells (leukocytes and monocytes) and associated tissue damages (Aviello and Knaus, 2017), the balance between commensal microbiota and host mucosal immune responses, and the promotional role of environmental factors (such as carbon monoxide and nitric oxide). We should also investigate how nanozymes influence the metabolism, morphology, behavior of immune cells, and signal pathways in the progression of IBD. Second, systematic pharmacokinetics and pharmacodynamics studies should be carried out. Third, the acute and chronic toxicity

studies should be performed to ensure the safety of nanozymes. Fourth, to demonstrate the superiority of nanozymes, a comprehensive comparison with currently used medications should be made. Fifth, larger animals rather than mice should be studied as a better model of IBD. The clinical use of nanozymes for IBD can be expected, when the listed challenges are fully addressed with the development of nanozyme research in the future.

**Compliance and ethics** The author(s) declare that they have no conflict of interest.

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## SUPPORTING INFORMATION

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