Catalytically potent and selective clusterzymes for modulation of neuroinflammation through single-atom substitutions

Haile Liu1,7, Yonghui Li1,7, Si Sun1,7, Qi Xin1, Shuhu Liu2, Xiaoyu Mu1, Xun Yuan3, Ke Chen1, Hao Wang1, Kalman Varga4, Wenbo Mi1, Jiang Yang5 & Xiao-Dong Zhang1,6 ✉

Emerging artificial enzymes with reprogrammed and augmented catalytic activity and substrate selectivity have long been pursued with sustained efforts. The majority of current candidates have rather poor catalytic activity compared with natural molecules. To tackle this limitation, we design artificial enzymes based on a structurally well-defined Au25 cluster, namely clusterzymes, which are endowed with intrinsic high catalytic activity and selectivity driven by single-atom substitutions with modulated bond lengths. Au24Cu1 and Au24Cd1 clusterzymes exhibit 137 and 160 times higher antioxidant capacities than natural trolox, respectively. Meanwhile, the clusterzymes demonstrate preferential enzyme-mimicking catalytic activities, with Au25, Au24Cu1 and Au24Cd1 displaying compelling selectivity in glutathione peroxidase-like (GPx-like), catalase-like (CAT-like) and superoxide dismutase-like (SOD-like) activities, respectively. Au24Cu1 decreases peroxide in injured brain via catalytic reactions, while Au24Cd1 preferentially uses superoxide and nitrogenous signal molecules as substrates, and significantly decreases inflammation factors, indicative of an important role in mitigating neuroinflammation.

1 Department of Physics and Tianjin Key Laboratory of Low Dimensional Materials Physics and Preparing Technology, School of Sciences, Tianjin University, 300350 Tianjin, China. 2 Beijing Synchrotron Radiation Facility (BSRF), Institute of High Energy Physics (IHEP), Chinese Academy of Sciences (CAS), 100049 Beijing, China. 3 School of Materials Science and Engineering, Qingdao University of Science and Technology, 266042 Qingdao, Shandong, China. 4 Department of Physics and Astronomy, Vanderbilt University, Nashville, TN 37235, USA. 5 School of Medicine, Sun Yat-sen University, 510060 Guangzhou, China. 6 Tianjin Key Laboratory of Brain Science and Neural Engineering, Academy of Medical Engineering and Translational Medicine, Tianjin University, 300072 Tianjin, China. 7 These authors contributed equally: Haile Liu, Yonghui Li, Si Sun. ✉email: xiaodongzhang@tju.edu.cn

https://doi.org/10.1038/s41467-020-20275-0

OPEN
Due to their exclusive catalytic activity and selectivity, artificial enzymes are exploited as promising tools for wide-reaching biomedical implications\(^1\)-\(^8\), particularly as advanced diagnostics\(^9\)-\(^10\) and therapeutics\(^11\)-\(^16\) of diseases. Earlier studies shed light on the oxidase- and peroxidase-like activities of noble metals\(^1\).\(^2\).\(^3\). Gold-based materials were unreserved to possess versatile enzyme-like activities, such as nuclease, glucose oxidase, peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD)\(^17\),\(^18\). The Michaelis–Menten constant (\(K_m\)) to the \(H_2O_2\) substrate of gold nanoparticles toward the POD enzymatic reaction is below 1 mM, but the catalytic activity is weak\(^19\). In contrast, Pt-based materials generally confer a high overall catalytic activity but it can only show a good \(H_2O_2\) substrate affinity when \(K_m\) is up to 16.7 mM\(^1\),\(^20\),\(^21\), and modulation of selective catalysis often needs to be purposely realized through rationally designed combination with other catalysts\(^22\). Meanwhile, metal oxides have also revealed great potentials as enzyme mimetics\(^23\),\(^24\). Typically, Fe\(_2O_3\) nanoparticles display the POD-like activity\(^23\),\(^24\) but are limited by their affinity to the \(H_2O_2\) substrate (\(K_m\) at ~154 mM) and a maximal reaction rate (5.9 \(\mu\)M/min) that do not meet expectations. Mn\(_2O_4\) nanoparticles concurrently exhibit SOD-, CAT-, and glutathione peroxidase (GPX)-like activities via the redox switch between Mn\(^{3+}\) and Mn\(^{4+}\) with a maximum reaction rate reaching 6–125 \(\mu\)M/min at nanomolar levels, which is unfortunately still inferior to natural enzymes\(^25\). Thus the development of catalytic artificial enzymes with exceptional activity, adequate selectivity, and satisfactory stability remains a major challenge for any foreseeable practical applications.

As is well known to all, most brain injuries involve enzyme-related catalytic processes and continuous neuroinflammation\(^26\)-\(^28\). However, it is largely unclear yet which specific catalytic route(s) can be selectively targeted to inhibit inflammatory responses, primarily because brain injuries simultaneously trigger various kinds of multi-enzymatic reactions between free radicals and numerous bioactive molecules\(^29\). Therefore, exploration of versatile artificial enzymes with different catalytic routes and desirable selectivity is beneficial to establish the relationship between oxidative stress and inflammation and to reveal the underlying molecular pathways of catalysis\(^30\)-\(^32\). Atomic-level catalysts suffice a viable solution for the unmet need of improved catalytic activity and precisely modulated selectivity in a controllable manner, with lots of Fe- and Pt-based single-atom nanozymes developed\(^33\)-\(^40\). In particular, Au contains excessive transition metal electronic states. The Michaelis–Menten constant (\(K_m\)) to the \(H_2O_2\) substrate of gold nanoparticles toward the POD enzymatic reaction is below 1 mM, but the catalytic activity is weak\(^19\). In contrast, Pt-based materials generally confer a high overall catalytic activity but it can only show a good \(H_2O_2\) substrate affinity when \(K_m\) is up to 16.7 mM\(^1\),\(^20\),\(^21\), and modulation of selective catalysis often needs to be purposely realized through rationally designed combination with other catalysts\(^22\). Meanwhile, metal oxides have also revealed great potentials as enzyme mimetics\(^23\),\(^24\). Typically, Fe\(_2O_3\) nanoparticles display the POD-like activity\(^23\),\(^24\) but are limited by their affinity to the \(H_2O_2\) substrate (\(K_m\) at ~154 mM) and a maximal reaction rate (5.9 \(\mu\)M/min) that do not meet expectations. Mn\(_2O_4\) nanoparticles concurrently exhibit SOD-, CAT-, and glutathione peroxidase (GPX)-like activities via the redox switch between Mn\(^{3+}\) and Mn\(^{4+}\) with a maximum reaction rate reaching 6–125 \(\mu\)M/min at nanomolar levels, which is unfortunately still inferior to natural enzymes\(^25\). Thus the development of catalytic artificial enzymes with exceptional activity, adequate selectivity, and satisfactory stability remains a major challenge for any foreseeable practical applications.

**Results**

**Structural properties of clusterzymes.** Exceedingly different from most previously reported nanozymes\(^1\),\(^4\),\(^3\), the as-developed 3-mercaptopropionic acid (MPA)-protected Au\(_{25}\) clusterzyme is stringently defined by its unambiguous atomic configuration and geometry structure (Fig. 1a). The hydrodynamic size of Au\(_{25}\) is determined to be 2.0 nm by dynamic light scattering (DLS), and the zeta potentials of all clusterzymes are around -35 mV, suggesting the ultrasmall size and good colloid stability (Supplementary Fig. 1). The characteristic absorption at 450 and 670 nm of Au\(_{25}\) is attributed to its unique interband transitions\(^42\),\(^43\), while a single-atom substitution of Cu or Cd induces a 2–3-nm minor shift, showing insignificant influence on optical properties (Fig. 1b and Supplementary Fig. 2). Electrospray ionization–mass spectrometry (ESI-MS) reveal a distinct \(m/z\) peak at ~2271, assigned to [Au\(_{25}\)MPA\(_{18}\)–3H]\(^{3+}\) (Fig. 1c). After one atom substitution by Cu and Cd, the characteristic \(m/z\) peak shifts to ~2226.6 and ~2243, respectively. The inductively coupled plasma-mass spectrometry (ICP-MS) confirms that the ratios of Cd and Cu to the total metal are 5 and 4%, respectively, further validating the successful introduction of single atoms (Supplementary Fig. 3). X-ray photoelectron spectroscopy (XPS) further confirms that Au (0) is the dominant state in all clusterzymes (Supplementary Fig. 4). To identify the precise spatial atomic configuration, extended X-ray absorption fine structure (EXAFS) spectra at the Au, Cu, and Cd edges were recorded (Fig. 1d, e and Supplementary Figs. 5 and 6). The \(L_3\) edges of Au in all clusterzymes have higher line intensities than the bulk standard Au foil. This is ascribed to larger surface area and alloying effects from partial oxidation with more \(d\)-band vacancies from nanoscale sizes and surface molecule-like interactions (Au(I)–thiolate). The characteristic absorption edges of Au clusterzymes were found at ~11,920 eV, which is assigned to the 2p – 5d electronic transition of Au suggesting a reduced population of unoccupied valence \(d\)-states. The increase in intensity in MPA-protected Au\(_{24}\)Cu\(_1\) and Au\(_{24}\)Cd\(_1\) indicates that the density of 5d electrons of Au is decreased by the one atom substitutions of Cu and Cd through the transfer of their \(4d\) electrons (Supplementary Fig. 5). The \(k\)-space oscillations of Au\(_{25}\) clusters and the Au foil are shown in Supplementary Fig. 5. The \(k\)-space of the Au foil exists in typical fcc oscillation patterns, which are apparently absent in all Au clusterzymes due to their small core sizes. Besides, we also investigated the X-ray absorption near edge structure (XANES) spectra of Cu and Cd foils as well as the corresponding atomic counterparts within clusterzymes, clearly displaying differences between single atoms and bulk metals (Supplementary Fig. 6). To further pinpoint the doping sites of Cu and Cd atoms, we performed fitting analysis on the EXAFS data of Cu and Cd. Figure 1d shows the \(R\) space of the EXAFS data of the Cu \(K\) edge in Au\(_{24}\)Cu\(_1\). It can be seen that there is only one major peak in the range of 1.6–5.0 Å. This peak roughly corresponds to the scattering path of photoelectron waves from the X-ray absorbing Cu atom to the neighboring S atoms of different shells, and IFEFIT program is used to fit this peak. The EXAFS parameters obtained after fitting are shown in Supplementary Table 1. The Cu-S coordination number (CN) obtained from the fitting is 1.9 ± 0.2 Å. This value is close to 2 Å, which may indicate that the replacement of Au\(_{25}\) by a Cu atom occurs at the oligomer site, consistent with previous work\(^44\). Similarly, the \(R\) space of EXAFS data on Cd \(L_3\) edge in Au\(_{24}\)Cd\(_1\) shows a peak in the range of 1.6–5.0 Å, and the fitted Cd-S CN is 2.3 ± 1.7 Å, which is close to the CN of the bond with S at the oligomer site of Au\(_{25}\), indicating that Cd atom substitution may occur at the oligomer site (Fig. 1e).\(^46\)

**Antioxidant properties of clusterzymes.** We tested the general antioxidant properties of all clusterzymes using the ABTS method (Fig. 2a) with reference to standard antioxidants trolox and anthocyanin. Negligible antioxidant activity was observed for pure MPA-protected Au\(_{25}\) clusterzyme. Single-atom substitutions with Cu or Cd in the structure, however, induce a dramatic increase in antioxidant activity with increasing concentrations (Fig. 2b). Among a variety of metals including Ag, Cu, Zn, Er, Pt,
and Cd, single-atom substituted candidates Au24Cu1 and Au24Cd1 show the highest activity, representing the optimal substituting elements and ratios (Supplementary Fig. 7). Time-course kinetics of Au24Cu1 and Au24Cd1 exhibit rapid responses to the substrate in seconds with high reaction rates (Fig. 2c). The quantitative results show Au24Cu1 and Au24Cd1 are 41 and 48 times higher in antioxidant activity than Au25, respectively (Fig. 2c). Compared with standard natural antioxidant controls, Au24Cu1 and Au24Cd1 are 137 and 160 times higher in activities than trolox and 7.5 and 9 times higher than ascorbic acid, respectively. The reaction rates of Au24Cu1 and Au24Cd1 at 10 and 14 μM/s are 8–11 times higher than Au25 or 38 and 51 fold higher than trolox, respectively. In addition, a parallel comparison with other elements, substitution with exactly one atom of Cu or Cd presents the foremost activity amongst all substituents (Supplementary Fig. 7d). Preceding studies have evidenced that atomically precise gold clusters, such as Au25 and Au38, are endowed with the oxidation catalytic activities25–28, but their antioxidant activities are rarely reported. Herein, we discovered its ultrahigh antioxidant activity with fast kinetics via atom substitution.

Enzyme-like properties of clusterzymes. The general catalytic profile of clusterzymes and the schematic diagram showing catalytic processes are displayed as in Fig. 3a, b. To pinpoint catalytic selectivity of these clusterzymes, we first investigated the GPx-like activity of Au25, Au24Cu1, and Au24Cd1 at the concentration of 10 ng/μL. Surprisingly, Au25 shows the strongest tendency toward GPx-like activity with a maximum reaction rate of 0.47 mM/min, higher than 0.34 mM/min for Au24Cu1 and 0.10 mM/min for Au24Cd1 (Fig. 3c), and also significantly higher than those of previously reported Mn3O4 nanoflowers (0.056 mM/min)51 and Co/PMCS (0.013 mM/min)52. The turnover frequency (TOF) value of Au25 calculated by the Michaelis–Menten equation is 320 min⁻¹, 4.7 times higher than Au24Cd1 (Supplementary Fig. 8). This result is interesting because metals are generally considered to have low GPx-like activity, but the high GPx-like activity of Au25 can be exploited to eliminate lipid peroxides and oxidative damages. The CAT-like activity of clusterzymes were studied at the concentration of 20 ng/μL as in Fig. 3d. The maximum reaction rate of Au25 is 0.074 mM/min, whereas the introduction of a Cu single atom gives rise to a 4.7-fold increase to 0.35 mM/min, suggesting its CAT-like catalytic preference. The calculated TOF value of Au24Cu1 for CAT-like activity is 116.7 min⁻¹ (Supplementary Fig. 9), which is significantly higher than that of Pd octahedrons (1.51 min⁻¹)53. The SOD-like activity of pure Au25 can only inhibit 31% of the substrate, while one Cd atom substitution considerably increases the inhibition rate to 89%, empowering SOD-like selectivity (Fig. 3e). The aforementioned results suggest enzyme-mimicking preferences of each individual clusterzyme: Au25 as GPx and Au24Cu1 and Au24Cd1 as CAT and SOD, respectively. The structures of clusterzymes before and after reaction with H2O2 suggest unchanged structures of the clusterzymes (Supplementary Figs. 10 and 11)54,55. Previous work mainly focused on the atomic substitutions of Au25 using noble metals for catalytic reactions of hydrogen and CO/CO256–59. Our work herein constructively hypothesized and demonstrated that Au25 can possess various unique enzyme-like activity modulated by single-atom substitution with non-precious metals like Cu and Cd, instead of Pt, in the geometric structure.

The corresponding specific scavenging of free radicals by the clusterzymes was further investigated. The scavenging of •OH free radical was investigated using electron spin resonance (ESR) by employing 5-tert-butoxy carbonyl-5-methyl-1-pyrroline N-oxide (BMPO) as the trapping agent. The ESR signal of •OH is strongly for the BMPO control, suggesting the presence of excessive •OH, while there is only a minor decrease after adding...
Au\textsubscript{25}, indicative of a weak scavenging efficiency for •OH (Fig. 3f). However, Au\textsubscript{24}Cu\textsubscript{1} almost completely diminishes all ESR signals (~100%), consistent with the observed best CAT-like activity as in Fig. 3d. Similarly, the scavenging of O\textsubscript{2}•\textsuperscript{-} by clusterzymes was also investigated (Fig. 3g). The ESR signal stays strong for the control and slightly decreases after addition of Au\textsubscript{25} and Au\textsubscript{24}Cu\textsubscript{1}, with surplus remaining residues. In contrast, the ESR signal of O\textsubscript{2}•\textsuperscript{-} almost disappears in the presence of Au\textsubscript{24}Cd\textsubscript{1} further validating its superior specialized SOD-like activity (Fig. 3e). Besides, we also tested the free-radical scavenging capability of the clusterzymes toward reactive nitrogen species (RNS) including •NO, ONOO\textsuperscript{-}, and DPPH•. Au\textsubscript{24}Cd\textsubscript{1} shows the most robust overall scavenging efficiency against DPPH• (Supplementary Fig. 12). The ESR reveals that Au\textsubscript{24}Cd\textsubscript{1} has the best scavenging capability toward •NO at a low concentration of 2.7 ng/μL, whereas Au\textsubscript{25} presents ignorable activity (Supplementary Fig. 13). Likewise, both Au\textsubscript{24}Cd\textsubscript{1} and Au\textsubscript{24}Cu\textsubscript{1} also manifest significantly higher scavenging efficiency toward ONOO\textsuperscript{-} than Au\textsubscript{25} (Supplementary Figs. 14 and 15). Au\textsubscript{24}Cd\textsubscript{1} is more selective against RNS than Au\textsubscript{25}Cu\textsubscript{1}, while Au\textsubscript{25} has insignificant catalytic activity. Thus it is rational to conclude that the high selectivity for enzymes and radicals originates from the single-atom substitutions of Cu and Cd, which induce redistribution of surface electrons and exert influence on electronic structures and states.

DFT calculations and the mechanism of catalytic selectivity. To reveal the catalytic mechanism, the density functional theory (DFT) was employed to investigate the catalytic selectivity and quantum properties. By exploring possible structures in the literature, we adopt the gold core of the well-known Au\textsubscript{25} clusters\textsuperscript{60} protected by ligands, which are still connected to the core via S atoms. To evaluate the catalytic behavior and the intermediate states during the chemical reactions, each ligand unit -SCH\textsubscript{2}CH\textsubscript{2}COOH is simplified to -SCH\textsubscript{3}. DFT optimization confirms the stability of the modeled cluster. Due to the symmetry of the gold cluster, all possible replacements of the guest metallic atoms fall into three categories as follows: oligomer, the surface of core, and core replacements. Figure 4a demonstrates the surface sites of the Au\textsubscript{13} core and the oligomer site replacement. The oligomer replacement is the common form that is extensively discussed in the literature, but DFT simulations indicate that the surface replacement may be another possibility. However, based on the coordination analysis in the experiment, the oligomer site replacement matches the EXAFS results, which yield a significantly lower CN than the surface replacement. With the optimized structure of the clusters, the associated CNs can be theoretically generated even within the clusters involved in the intermediate structures during the catalytic process. The averaged simulated CN values agree with the experimental values that confirm the oligomer replacement
Although more theoretical investigations on the surface replacements can be found in the appendix, we focus on the oligomer replacement and the associated catalytic efficiency.

Unlike the surface replacement that may cause the expansion of the core, the oligomer replacement causes the oligomer bending. It is different from the normal S-Au-S chain, which aligns in a (nearly) straight line (Supplementary Fig. 20). The doped Cu shrinks the S-X-S chain while the doped Cd extends it. Compared with the typical bond length of S-Au at 2.3 Å, S-Cu and S-Cd bonds are 2.2 and 2.55 Å, respectively, as shown in Fig. 4b and Supplementary Fig. 21. With the bent chain, the distances between Cu/Cd atoms to the surface of the core are comparable, around 3.1 Å. The similarity between the Cu and Au atoms guarantees that the binding of S-Cu-S is so “firm” that the relative positions of Cu to S atoms can be hardly changed by the dynamics during the catalytic procedures, which are discussed extensively below. In contrast, the relative position of the doped Cd atom may be significantly affected by the local environment such as the adhesion of small chemical units (Supplementary Figs. 22 and 23).

We observed the excellent performance of the clusterzymes in both CAT and SOD reactions with the reaction pathways summarized in Fig. 4c. The CAT reaction usually refers to the catalytic degradation of hydrogen peroxide, and the decomposition mechanism of H₂O₂ may involve multiple chemical stages (Fig. 4d, e). For the process of SOD, we assumed that the clusters were involved in similar mechanisms to the general catalytic scheme of SOD reaction. It is worth noting that the release of oxygen completes the CAT process, while the SOD process occurs simultaneously, and the two processes are mutually permeated. The reduced cluster, Cluster(I), may also be involved in both CAT and SOD processes, which depends on the concentration of different components.

Inspired by the Arrhenius equation, we performed the search of transition states and ground states of various types of molecules and ions to estimate the activation energies and evaluated the catalytic efficiencies. The energy profiles in Fig. 4f, g agree with the behaviors of the clusterzymes in our experiments.
In a series of reactions with multiple steps, the reaction rate is dominated by the slowest step, i.e., the transition with the largest activation energy. Such a feature can be seen in the first part of the catalytic SOD process by Au$_{24}$Cu$_1$. The ground state of the electrons in the corresponding intermediate structure is a triplet state suggested by DFT simulations. It indicates that the high activation energy of 131.2 kJ/mol is related to the spin matching issues, which can be selective to the spin of superoxide ions. In the CAT processes, the critical step is related to the decomposition of (cluster…OOH)$_2^+$, in which the Au$_{24}$Cd$_1$ exhibits higher activation energy (71.3 kJ/mol) that reduces the efficiency. The simulations clearly explain the SOD–CAT-selective behaviors of the doped clusterzymes.

Our results of DFT simulations show some insights of the catalytic mechanisms. Assume in typical clusters, a substituted atom may turn into an active site itself to be involved in the catalytic process, which may be accompanied by changes in the geometry. Herein, we named two mechanisms as SA (simple adhesion) and MA (bond modulated adhesion) correspondingly. The distances between the adsorbed molecule/ion and metal atoms designate the roles of the substituted atom.
The SA mechanism is mainly seen in Au$_{24}$Cu$_1$. Due to the firmness of the S-Cu-S oligomer, the Cu atom is relatively rigid (Fig. 4f, g). The SA mechanism is also seen in the first step of SOD process catalyzed by Au$_{24}$Cd$_1$. The catalytic process includes the distance change and orientation change of small units. Significant changes in the distance between the active site (doped atom) and the small units are seen in most of the SOD processes. In contrast, the orientation change is the main character in most of the CAT processes.

The MA mechanism is seen in Au$_{25}$Cd$_1$ in the second stage (Cluster(I)) of the SOD processes and most of the CAT processes. The bond modulation refers to the position change of the Cd atom, which may deviate from the oligomer plane until a third S atom from another oligomer stops it. Thus the S-Cd bonds are changed significantly (Fig. 4f, g). The characteristics of transition and intermediate states involve the rotation of the superoxide ion (or oxygen molecule) and the position adjustment of the doped Cd atom. To be more precise, the motion of the Cd atom is along the perpendicular direction of the oligomer plane. Once a small unit joins the doped cluster to form an intermediate structure, the Cd atom sometimes leaves the oligomer plane. Therefore, the CN value of the Cd atom is larger when the Cd atom becomes the neighbor of three S atoms. When the Cd atom starts from its original state (S-Cd = 2.55 and 2.55 Å), passes its transition state (S-Cd = 2.63 and 2.85 Å), and arrives at the intermediate state (S-Cd = 2.57 and 2.60 Å), the angular motion is terminated (Supplementary Fig. 24). During such a process, the distance between the attached oxygen atoms is slightly expanded toward the normal distance of oxygen molecules, which indicates the completion of the entire catalytic procedure (Supplementary Tables 5 and 6). A similar procedure for the Au$_{24}$Cd$_1$ can be observed at the adhesion of OOH- at the first stage of CAT process. Such a unique process allows the doped Cd atom to be an active site that can be self-modulated in a wide spatial range compared to the firm Cu atom. This may explain its good performance in the SOD process.

**Modulation of neuroinflammation.** To reveal the biological activity of clusterzymes, the cell toxicity for different nerve cell lines (HT22, BV2, and MA-c) were measured by the 3-[4,5- dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay (Fig. 5a and Supplementary Fig. 25), showing that Au$_{25}$, Au$_{24}$Cu$_1$, and Au$_{24}$Cd$_1$ present acceptable biocompatibility. Cell survival of H$_2$O$_2$-stimulated neuron cells was performed with the incubation of Au$_{25}$, Au$_{24}$Cd$_1$, or Au$_{24}$Cu$_1$. As shown in Fig. 5b, the clusterzyme treatment could improve the viability of neuron cells. To explore the correlation between the oxidative stress and the neuron viability, reactive oxygen species (ROS), especially •OH and O$_2$•−, were quantified and detected by a FACS flow cytometer and a fluorescence microscope using hydroxyphenyl fluorescein (HPF) and dihydroethidium (DHE) fluorescence probes, respectively. The H$_2$O$_2$ stimulation significantly elevates the fluorescence signal, indicating the presence of excessive amount of •OH and O$_2$•− (Fig. 5c-h). All clusterzymes decrease the ROS signals, with Au$_{24}$Cu$_1$ showing the best clearance efficiency against •OH (Fig. 5c, e, g) and Au$_{24}$Cd$_1$ displaying the best clearance capability for O$_2$•−, suggesting their individual selectivity (Fig. 5d, f, h). Meanwhile, mouse models of traumatic brain injury (TBI) were used to examine the in vivo effects of clusterzymes. As shown in Fig. 5i-l, the indicators of malondialdehyde (MDA), H$_2$O$_2$, SOD, and GSH/GSSG in the TBI group are relatively severe at day 1 post injury but are slightly alleviated 3 days post injury and are further improved slightly 7 days post injury. Therefore, the decrease in SOD and GSH/GSSG levels from TBI can be well rescued by clusterzymes with prominent recoveries 7 days after treatment (Fig. 5i, j). Comparatively, Au$_{24}$Cd$_1$ induce a better recovery in SOD than Au$_{24}$Cu$_1$, which correlates well with their in vitro SOD-like activity (Fig. 3). As the by-products of the oxidative stress, lipid peroxides and H$_2$O$_2$ show higher accumulations in the brain following TBI, resulting in severe oxidative damage (Fig. 5k, l). Both Au$_{24}$Cu$_1$ and Au$_{24}$Cd$_1$ significantly inhibit the production of these harmful molecules, while Au$_{25}$ barely alters the TBI-induced increase. These results are conceivable because O$_2$•− is known to be continuously produced by immediate injuries at the early stage, followed with subsequent production of lipid peroxides and H$_2$O$_2$. With regard to Au$_{24}$Cd$_1$, it can recover the diminished SOD in the first place due to its high catalytic selectivity for O$_2$•− and then sustain the continuous decrease of lipid peroxides and H$_2$O$_2$ as the secondary catalytic options. In contrast, Au$_{24}$Cu$_1$ is primarily prone to increase the levels of lipid peroxides and H$_2$O$_2$ at the early stage due to its preference for CAT-like activity and •OH, but these molecules are intermediate products at relatively low concentrations after TBI onset, and consequently it accounts for the increasing clearance capability in the long term.

Finally, the effects of clusterzymes on neuroinflammation were examined. From the western blots and the relevant quantification analysis (Fig. 6a, d), the expression levels of interleukin (IL)-1β and IL-6 are significantly upregulated following TBI 1 day post injury, indicative of strong local inflammations. Au$_{24}$Cd$_1$ sharply downregulates IL-1β and IL-6 levels, suggesting the anti-neuroinflammation effect. In comparison, Au$_{25}$ only shows minor downregulation. Similarly, TBI leads to significant upregulation of tumor necrosis factor-α (TNFa) 1 day post injury, but Au$_{25}$Cu$_1$ can significantly downregulate the expression of TNFa, presenting superior efficacy over Au$_{25}$ and Au$_{24}$Cd$_1$ (Fig. 6a). Although the expression levels of these inflammation cytokines induced by TBI are gradually suppressed by autoimmunity in the vehicle control group over time, especially 7 days post injury, there are still significant differences in IL-1β and IL-6 levels between the Sham and TBI groups (Fig. 6b-d). However, the clusterzyme treatment results in cytokines close to the normal level, indicating a better suppression effect on neuroinflammation. Enzyme-linked immunosorbent assay (ELISA) further validated the immunoblotsing results that Au$_{24}$Cd$_1$ and Au$_{24}$Cu$_1$ are capable of decreasing the inflammatory cytokines in brain tissues such as IL-1β, IL-6, and TNFa, while Au$_{25}$ does not significantly alter the inflammatory cytokine patterns (Fig. 6e-g). Au$_{24}$Cd$_1$ can eliminate IL-1β- and IL-6-associated inflammatory responses, while Au$_{24}$Cu$_1$ has a better effect on reduction of TNFa, indicating their relevant selectivity toward modulation of neuroinflammation. Finally, immunofluorescence staining of cerebral cortex harvested from mice also shows that Au$_{24}$Cd$_1$ and Au$_{24}$Cu$_1$ can remarkably decrease the TBI-elevated expression levels of IL-1β, IL-6, and TNFa (Fig. 6h, i and Supplementary Figs. 26 and 27), therefore alleviating neuroinflammation. Colocalization studies with markers for neurons (NeuN), microglia (Iba-1), or astrocytes (glial fibrillary acidic protein (GFAP)) were performed in injured cortex on day 3 post injury. Figure 6h, i reveal that IL-1β is mainly produced by microglia after TBI, similar with IL-6 and TNFa (Supplementary Figs. 26 and 27). In addition, quantitative analyses of the number of positive cells show that massive microglia and astrocytes are activated, and many neurons are depleted after TBI (Fig. 6j). With the clusterzyme treatment, most of these nerve cells are rescued. Meanwhile, the morphology of TBI-activated astrocytes can be recovered to near normal levels after treatment with clusterzymes (Supplementary Fig. 28), and the neuroinflammatory responses are also prevented likewise by verified histology (Supplementary Figs. 29–32). The clusterzymes can also restore the TBI-induced
Moreover, behavioral tests were studied by the Morris water maze (MWM). As shown in Supplementary Fig. 34a, b, all the mice apparently learned the task during the acquisition phase of days 13–17 and 28–31, while the distance traveled and latency to hidden platform with Au24Cu1 and Au24Cd1 treatment obviously decreased. For the probe trial on days 18 and 32 (Supplementary Fig. 34c, d), the percentage time in the missing platform quadrant and the number of platform crossings were significantly reduced in the TBI group but almost return to the normal level after Au24Cu1 and Au 24Cd1 treatment. These results reveal trends in the improvements of learning ability and spatial memory with Au24Cu1 and Au24Cd1 treatment. In addition, we systematically studied the pharmacokinetics and toxicology of clusterzymes. It can be seen that the clusterzymes accumulated in major organs can be removed by the kidney (urine) and liver (feces). After 48 h, ~80% of the total dose can be excreted, and most of it is excreted through the kidney (>70%) (Supplementary Fig. 35). No significant changes in organs or blood chemistry or hematology are found, suggesting that renal-clearable clusterzymes do not cause significant biological toxicity in vivo (Supplementary Figs. 36–38). Artificial enzymes have persistently been shown to exhibit multiple enzyme-like catalytic activities with a diversified class of materials15. Low catalytic activity as compared to natural enzymes, however, is one of the most noticeable disadvantages due to limited electron transfers at atomic levels15. The rationally designed clusterzymes with single-atom substitutions overcome such barriers with antioxidative activity nine times higher than that of natural enzymes.

Fig. 5 Oxidative stress levels in vitro and in vivo before and after treatment of clusterzymes. a HT22 cell viability of clusterzymes (n = 5 per group, data are presented as mean ± SD). b HT22 cell viability in the presence of H2O2 with or without treatment of clusterzymes as determined by MTT assays (n = 6 per group, data are presented as mean ± SD). Fluorescence quantification of cell staining for c, e •OH and d, f O2•− by flow cytometry (n = 3 per group). Data are presented as mean ± SD and compared with the Con and H2O2 groups, analyzed by one-way ANOVA with two-sided LSD test (adjusted p values are shown). Fluorescence microscopic images of intracellular g •OH (green) and h O2•− (red) levels induced by 100 μM H2O2 with or without clusterzymes treatment, stained by HPF and DHE probes, respectively. It can be seen that Au24Cu1 has a better scavenging ability for •OH and Au24Cd1 shows better specificity for O2•−, suggesting their individual selectivity for •OH and O2•− respectively. i–l Indicators for oxidative stress, including SOD, GSH/GSSG, MDA, and H2O2 of TBI mice with or without treatment of clusterzymes 1, 3, and 7 days post injury (n = 5 per group). Data are presented as mean ± SEM and compared with the Sham and TBI groups, analyzed by one-way ANOVA with two-sided LSD test (adjusted p values are shown). Experiments were repeated independently g, h three times with similar results.
of anthocyanin, which is known to be one of the most reactive antioxidant molecules in nature. Besides, unlike the structurally ambiguous traditional artificial enzymes, the definitive molecular structures of clusterzymes are accurately elucidated, allowing us to distinguish the catalytically active sites and scrutinize the electronic structures and reaction energies. As a result, the substituting single atoms can be arranged into a specific spatial location of the clusterzyme freely, thus tuning electronic structures and affecting the catalytic activity. Meanwhile, the interactions between host atoms (i.e., Au) and the introduced substituting atoms (i.e., Cu or Cd) can induce coupled electron states and in turn influence the catalytic selectivity. In our work, the GPx-, SOD-, and CAT-like catalytic selectivity were assigned to Au25, Au24Cd1, and Au24Cu1, respectively, via modulated bond lengths to the active center, and thus it is conceived that such a platform of clusterzymes will generate various selectivity against different molecules. By employing the three catalytically selective clusterzymes, we successfully established the relationship between oxidative stress and neuroinflammation, demonstrating the importance of O2•− and long-term benefits in TBI. Specifically, Au24Cd1 can significantly mitigate the neuroinflammation via inhibiting IL-1β and IL-6, while Au24Cu1 differentially reduces neuroinflammation by inhibiting TNFα, showing selectivity against anti-neuroinflammation. Meanwhile, due to the innate ultrasmall size of clusterzymes, it can penetrate the kidney barriers and be excreted by renal, avoiding long-term hepatotoxicity and multi-organ injuries. Therefore, the clusterzymes are presumably influential as a biomedicine, especially in the field of neuroscience.

Discussion

In summary, we report a systemic single-atom substitution approach to fabricate artificial enzymes on the basis of MPA-protected Au25 clusters, namely clusterzymes. The clusterzymes show the ultrahigh antioxidant activity up to 137–160 times the reaction rate of the natural enzyme, demonstrating their potential for the treatment of oxidative stress-related diseases. The substitution of single atoms into the clusterzyme framework allows for the tuning of electronic structures and reaction energetics, providing a versatile platform for the design of artificial enzymes with tailored catalytic properties.

Fig. 6 Inflammation levels in brain tissues after clusterzyme treatment. a–c Western blotting for IL-1β, IL-6, and TNFα in the brain tissues 1, 3, and 7 days post TBI after treatment (n = 3 per group), respectively. d Western blotting quantitative analysis of inflammatory factors at different time points (n = 3 per group). All the samples were derived from the same experiment and blots were processed in parallel. Data are presented as mean ± SEM and compared with the Sham and TBI groups, analyzed by one-way ANOVA with two-sided LSD test (adjusted p values are shown). It can be seen that Au24Cd1 can rapidly and significantly reduce the upregulated inflammatory cytokines of IL-1β and IL-6 after brain injury, while Au24Cu1 has a better ability to reduce the expression of TNFα. e–g ELISA quantitative analysis of IL-1β, IL-6, and TNFα levels in brain tissues on days 1, 3, and 7 with and without clusterzyme treatment (n = 5 per group), respectively. Data are presented as mean ± SEM and compared with the Sham and TBI groups, analyzed by one-way ANOVA with two-sided LSD test (adjusted p values are shown). h Immunofluorescence co-staining of IL-1β and microglia (Iba-1), astrocytes (GFAP), or neurons (NeuN) in injured cortex 3 days post injury with or without clusterzyme treatment. Quantitative analysis of i the number of IL-1β expression in different positive cells and j the pixels density of Iba-1/NeuN/GFAP cells in the injured cortex with or without clusterzyme treatment (n = 3 per group). Data are presented as mean ± SEM and compared with the Sham and TBI groups, analyzed by one-way ANOVA with two-sided LSD test (adjusted p values are shown). Experiments were repeated independently a–c twice and h three times with similar results.
higher than the natural trolox. Moreover, the catalytic selectivity toward GPx, CAT, SOD, and nitrogen-related signaling molecules can be fine-tuned by single-atom substitutions. DFT calculations conclude that reaction pathways are modulated by the single active site of Au24Cd1 and Au24Cu1 at bond lengths. The biological results show that Au24Cd1 preferentially decreases IL-1β and IL-6, while Au24Cu1 tends to decrease TNFα, indicative of their different selectivity for modulating alleviation of neuroinflammation.

**Methods**

**Materials.** All chemicals are commercially available with the highest purity and used without further treatment. Gold chloride (HAuCl4·3H2O) was purchased from Sigma-Aldrich; sodium hydroxide (NaOH), sodium borohydride (NaBH4), copper nitrate (Cu(NO3)2), cadmium nitrate (Cd(NO3)2), and MPA were purchased from Aladdin. Ultrapure water (18.2 MΩ·cm) was used for all the experiments.

**Materials’ preparation.** The gold nanoclusters were synthesized according to the previous literature3. In detail, aqueous solutions of HAuCl4 (20 mM, 0.25 mL) and MPA (5 mM, 2 mL) were added to water (2.35 mL) and stirred at room temperature for 5 min. Then an aqueous NaOH solution (1 M, 0.3 mL) was added to the reaction solution, followed by the addition of 0.1 mL of an NaBH4 solution (prepared by dissolving 43 mg of NaBH4 powder in 10 mL of 0.2 M NaOH solution). The whole reaction was carried out in the dark, and Au24MPA52 was collected after stirring at room temperature for 3 h, and the final reaction solution aged at 4 °C for 12 h. The syntheses of various metal-substituted Au52-SG52 were also based on the same method. The only difference was that the Au atoms in HAuCl4 (20 mM, 0.25 mL) were replaced by various metal ions (Cu2+, Cd2+ and Cu3+ at 4% molar ratio: Au:M = 4:1). For further purification of nanoclusters, we used ultrafiltration tubes of 3 and 10 k at 3500 rpm/min for ultrafiltration to remove smaller organic ligands and larger-sized clusters and lyophilized to obtain the purified product for further testing and application.

**Materials’ characterization.** Ultraviolet–visible (UV-vis) absorption spectra were recorded on Shimadzu 3600 UV-vis-NIR spectrophotometer. ESI-MS were acquired on Bruker microTOF-Q system. XANES along with EXAFS analyses were acquired on Bruker microTOF-Q system. XANES and EXAFS spectra were processed using ARTEMIS of programs of IFEFFIT were used for processing data of XANES and EXAFS75. Ultraviolet–visible (UV–vis) spectra of various clusters was recorded on Shimadzu 3600 UV-vis-NIR spectrophotometer. ESI-MS were tested and provided by Beijing Synchrotron Radiation Facility. The module ARTEMIS of programs of IFEFFIT were used for processing data of XANES and EXAFS47,50. Clusterzymes were prepared by mixing HAuCl4 and MPA Cu(NO3)2, Cd(NO3)2, and MPA were purchased from Aladdin. Ultrapure water (18.2 MΩ·cm) was used for all the experiments.

**Antioxidant and free radical scavenging tests**

**Total antioxidant capacity test (ARTS rapid method).** The total antioxidant capacity (T-DOC) of clusterzyme and contrast (trolox and anthocyanin) was determined by the rapid ARTS method using the T-DOC Assay Kit (S0121, Beyotime). Please refer to the specification for specific sampling methods. The antioxidant capacity was evaluated by measuring the absorption value at 414 nm. In the process of reaction kinetic analysis, we adjusted the concentration of different concentration of ABTS+ (the molar extinction coefficient of ABTS+: ε414 nm = 3.6 × 10^3 mol·L^−1·cm^−1) by changing the concentration of H2O2. The reaction kinetic analysis process was reflected by the change of absorbance at 414 nm monitored by the UV–vis spectrophotometer under kinetic mode. The steady-state kinetic parameters were determined by varying the concentration of ABTS+ in the presence of clusterzymes (5 μg/μL). The maximum reaction velocity (V_max) and Michaelis–Menten constant (K_m) were calculated using the Lineweaver–Burk equation.

**RNS scavenging test.** The RNS scavenging capacity of clusterzymes was performed for 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). The scavenging capacity of free radicals was evaluated by measuring the absorption wavelength at 510 nm. Briefly, 50 μM DPPH+ and 5 μg/μL clusterzyme were dissolved in a mixture of dimethyl sulfoxide and water (1:40). The changes of absorption spectra with time in the range of 300–1000 nm were determined.

**Enzyme-like activity test**

**CAT-like test.** CAT-like activity of clusterzymes was determined by two methods. First, according to the unique absorption peak of H2O2 at 240 nm, the optical density decreases with the decomposition of H2O2, and the extinction coefficient of H2O2 (43.6 mM–1·cm^−1 at 240 nm) was used to calculate its activity. The reaction solutions contained 53 μM H2O2 and 10 ng/μL of clusterzyme in 200 μL PBS. H2O2 was used with or without 50 ng/μg clusterzyme for 30 min to obtain the photos of H2O2 decomposition in the centrifuge tube. Another method was to measure the kinetics of the CAT-like activity using the Dissolved Oxygen Meter (HACH HQ40d, US) with LDO101 probe. First, the solubility of O2 in solution was reduced to 0.6 mg/L by continuous infusion of Ar into 5 mL PBS solution. Then, different concentrations of H2O2 (50–1000 mM) and 20 ng/μL of clusterzymes were added to the system to monitor the solubility change of O2 every 10 s. The interference deducted from each set of experiments is the effect of H2O2 self-decomposition under the same conditions. The maximum reaction velocity (V_max) and Michaelis–Menten constant (K_m) were calculated using the Lineweaver–Burk equation by the Origin 9.0 software.

**SOD-like test.** The SOD-like activity of clusterzymes was tested according to the description in the SOD Activity Assay Kit. After adding clusterzymes (0–10 ng/μL) of different concentrations, the absorbance changes at 560 nm were monitored with UV-vis spectrophotometer to further evaluate SOD-like activity.

**DFT calculations.** To investigate the catalytic effect, we focus on the energy profile of the clusterzymes in their intermediate structures. The adsorption energy that describes the energy change of an adsorbate when being attracted by a cluster is calculated by the following equation:

\[
E_{\text{ads}} = E_{\text{catalyst}} - \left( E_{\text{ads}} + E_{\text{cat}} \right).
\]
The \(E_{\text{act}}\) and \(E_{\text{ads}}\) denote the energy of the cluster and the adsorbate, respectively, while the \(E_{\text{cat}}-E_{\text{act}}\) is the energy of the intermediate structure. In addition, we also simulate the activation energies, which are the differences of the energies between the transition states and energetically stable states.

\[
E_{\text{bulk}} = E_{\text{cat}} - E_{\text{act}} - E_{\text{ads}}.
\]

The transition states, which are used to estimate the activation energy barriers that corresponds to the saddle points on an energy surfaces \(E_{\text{cat}}-E_{\text{act}}\), are searched using Broyden algorithm.

The CAT process. A CAT reaction usually refers to the catalytic degradation of hydrogen peroxide. The total reaction is

\[
2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2.
\]

In our experiment, we observed outstanding performance of the clusterzymes as the CATs. The mechanism of the decomposition of \(\text{H}_2\text{O}_2\) may involve multiple steps and multiple paths. Considering the catalytic mechanism of copper (II), we propose the clusterzyme initiate the reaction in the following steps:

\[
\text{cluster}^{++} + \text{H}_2\text{O}_2 \rightarrow (\text{cluster} \cdots \cdot \text{OOH})^{++} + \text{H}^+.
\]

Then the intermediate structure, cluster \(\cdots \cdot \text{OOH}^{+}\), decomposes into three pieces: a cation, a superoxide ion, and the original cluster with an extra electron, Cluster (I):

\[
(\text{cluster} \cdots \cdot \text{OOH})^{+} \rightarrow \text{cluster}^{+} + \text{O}_2^{+} + \text{H}^+.
\]

The superoxide ion may be involved in a process of hydroxyl scavenging (Eq. 6) or follow the SOD reaction path. The reduced cluster can cause a disproportionation-like process in which the hydrogen peroxide is not equally divided:

\[
\text{cluster}^{+} + \text{H}_2\text{O}_2 \rightarrow (\text{cluster} \cdots \cdot \text{OH})^{+} + \text{H}_2\text{O}.
\]

The hydroxyl radicals produced (Eq. 6) may be cleaned by the process in Eq. (6). The superoxide ions produced in Eq. (5) are partially involved in the SOD reaction as we shall discuss in the next part.

The SOD process. For the process of SOD, we assume that the clusters are involved in similar mechanisms to the general catalytic scheme of SOD reaction [Eq. (6)]:

\[
\text{O}_2^{+} + \text{Cluster}^{++} \rightarrow \text{O}_2 + \text{Cluster}^+,
\]

\[
\text{O}_2^{+} + 2\text{H}^+ + \text{Cluster} \rightarrow \text{H}_2\text{O}_2 + \text{Cluster}^{++}.
\]

Equation (9) shows the release of oxygen, which complete the CAT process, and it is also the initialization of SOD process. The reduced cluster, Cluster (I), may also be involved in both CAT and SOD process, which depends on the concentration of different components. Equations (9) and (10) are combined to the total reaction:

\[
2\text{O}_2^{+} + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2.
\]

Please note that Eq. (10) is not the end of the reaction, but the end of SOD. The hydrogen peroxide can be decomposed by the CAT process mentioned above. Furthermore, the superoxide ions may react with the hydrogen peroxide as the following reaction:

\[
\text{O}_2^{+} + \text{H}_2\text{O}_2 \rightarrow \text{OH} + \text{OH} + \text{O}_2.
\]

The hydroxyl radicals are produced in such reaction, but the reaction is slow to be biologically significant. So in this work, we do not consider such reaction and the production of hydroxyl radicals.

In vitro experiments. Mouse hippocampal neuronal HT22 cells were obtained from the Institute of Radiation Medicine, Chinese Academy of Medical Sciences, and Peking Union Medical College and employed in all the cellular experiments. Mouse microglia BV2 cells and mouse astrocytes-cerebral MA-c cells were obtained from Tianjin Huanhu Hospital. Cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM) ( Gibco), supplemented with 10% fetal bovine serum (FBS, BI) at 37°C with 5% CO\(_2\). In all, 100 U/ml penicillin and 100 mg/ml streptomycin sulfate (Solomo) were applied according to the growth state.

Cytotoxicity assay. HT22 cells (2 × 10\(^4\)), BV2 cells (3 × 10\(^3\)), and MA-c cells (4 × 10\(^3\)) were seeded in 96-well plates filled with 0.01 M PBS (Gibco) at the border in 100 µL medium overnight. The culture medium was replaced by different doses of Au25, Au24Cu1, or Au24Cd1 dissolved in the DMEM, and then cells were incubated for another 24 h. Wells were replaced by 0.01 M PBS once, and the medium were replaced by fresh culture medium with serum-free DMEM. Cell cytotoxicity was determined by SPSS 19 MTT assay at the MTT concentration of 5 mg/mL for 2.5 h and detected at optical density (OD) 490 nm.

Cell viability. HT22 cells (2 × 10\(^3\)) were cultured in the 96-well plate in 100 µL culture media. When reaching roughly 60% confluence in each well, cells were treated with 100 µM H\(_2\)O\(_2\) for 6 h. Then the culture media were substituted by fresh media containing Au25, Au24Cu1, or Au24Cd1 at different doses, and cells were incubated overnight. The plates were washed with PBS and incubated with 5 mg/mL MTT for 2.5 h. Cell viability was determined by MTT assay and analyzed at OD 490 nm.

Measurement of intracellular oxidative stress. HT22 cells (2 × 10\(^3\)) were cultured into 6-well plate in 2 mL culture medium. HT22 cells were grown to 60% confluence and treated for 6 h under 100 µM H\(_2\)O\(_2\) conditions. The solution was replaced by fresh culture medium with 10% FBS containing 6 ng/µL Au25, Au24Cu1, or Au24Cd1 and cells were incubated for another 18 h. Then cells were incubated with 25 µM DHE (Beyotime, S0063) for 25 min at 37°C in the dark to determine O2•− level. After 25 min, the culture medium containing DHE was removed, and the wells were washed with 0.01 M PBS. For •OH levels, the cells were incubated with 50 µM H2O2 (Sigma-Aldrich, H4290) for 25 min at 37°C in the dark. Intracellular oxidative stress was captured by using a fluorescence microscope (EVOS, AMG), collected data by an FACS flow cytometer (BD AccuriTM C6), and used Flowjo 10.6.2 for quantitative analysis of free radicals. Cells were gated based on size and granularity by forward and side scatter (SSC-A versus FSC-A). Then cell gate was analyzed for fluorescence intensity to determine the scavenging ability of clusterzymes. Among samples, control is recognized as the negative group, and H\(_2\)O\(_2\) is referred as the positive group.

In vivo treatment. All animal procedures were approved by the Institute of Radiation Medicine, Chinese Academy of Medical Sciences, and Peking Union Medical College. Procedures were applied to minimize the number of animals used and the pain mice suffered.

Animal models. Male C57BL/6 mice were purchased from SPF (Beijing) Biotechnology Co., Ltd. Mice were housed in a constant temperature (21–23°C) and humidity environment (45–60%) with a 12-h light–dark cycle. Food and water were available ad libitum. Surgery was performed after 1 week of transportation to adapt to the environment. Controlled cortical impact (CCI) models were conducted on adult male C57BL/6 mice (7–9 weeks, 21–23 g). C57BL/6 mice were assigned to the Sham (n = 37 per group), TBI, TBI/50 mg/kg dissolved in 0.01 M PBS. An injection volume of 200 μL was used for the Sham, TBI, and other groups. All mice were marked, classified, and put into the cages (5 mice/cage) under a specific pathogen-free-level environment. Food and water were available ad libitum. Surgery was performed after 1 week of transportation to adapt to the environment. Controlled cortical impact (CCI) models were conducted on adult male C57BL/6 mice (7–9 weeks, 21–23 g). C57BL/6 mice were assigned to the Sham (n = 37 per group), TBI, TBI/50 mg/kg dissolved in 0.01 M PBS. An injection volume of 200 μL was used for the Sham, TBI, and other groups. All mice were marked, classified, and put into the cages (5 mice/cage) under a specific pathogen-free-level environment. Controlled cortical impact (CCI) models were conducted on adult male C57BL/6 mice (7–9 weeks, 21–23 g). C57BL/6 mice were assigned to the Sham (n = 37 per group), TBI, TBI/50 mg/kg dissolved in 0.01 M PBS. An injection volume of 200 μL was used for the Sham, TBI, and other groups. All mice were marked, classified, and put into the cages (5 mice/cage) under a specific pathogen-free-level environment.

Inflammation injury and intravenously injected 0.01 PBS for 10 min. Sham-injured groups (control) received the same craniotomy and intravenous injection with enhanced BCA Protein Assay Kit (Beyotime, P0010). Oxidative stress-related factors MDA, SOD, GSH/GSSG, and H\(_2\)O\(_2\) were detected with lipid peroxidation MDA Assay Kit (Beyotime, P0004).
benzidine tetrahydrochloride hydrate was utilized for detection. Slides were then rinsed three times and 3,3-diaminobenzidine (S2110, Solarbio, China) and photographed with a fluorescence microscope. The secondary antibody information is as follows: anti-TNF-α (EVOS, AMG). For immunohistochemistry staining, the primary antibody information is as follows: anti-TNFα antibody (1:150, Abcam, ab183218), anti-IL-2 antibody (1:200, Bioss, bs-0782R), anti-IL-1β (1:200, Bioss, bs-0812R), antibody anti-NeuN antibody (1:800, GeneTex, GTX00387), anti-Iba1 antibody (1:300, Abcam, ab48004), and anti-GFAP (1:400, Abcam, ab90601). The primary antibody was removed and slides were rinsed with PBS three times 3 min each. Indicated fluorescence-labeled secondary antibodies were added and incubated at room temperature for 1 h in dark. The secondary antibody information is as follows: CoraLite488-conjugated Anti-Goat IgG H&L (Alexa Fluor 647) (1:1000, Abcam, ab150171), Donkey Anti-Goat IgG H&L (Alexa Fluor® 647) (1:1000, Abcam, ab150177), Donkey Anti-Goat IgG H&L (Alexa Fluor® 647) (1:1000, Abcam, ab150131). Finally, slides were mounted with anti-fade mounting medium with 4,6-diamidino-2-phenylindole (S2110, Solarbio, China) and photographed with an fluorescence microscope (EVOS, AMG). For immunohistochemistry staining, the primary antibody information utilized is as follows: anti-TNFα antibody (1:200, Abbkine, ABP0127), anti-IL-6 antibody (1:100, Bioz, bs-1008R), anti-IL-1β (1:200, Abcam, ab259323). After rinsing with PBS, the biotinylated secondary antibody was initially applied for 30 min, after reaction enhancer in Universal Two-step Detection Kit (ZSGB-BIO, pv9000) was used for additional 30 min. 3,3-diaminobenzidine tetrahydrochloride hydrate was utilized for detection. Slides were then counterstained with hematoxylin to stain nuclei. Samples were captured by microscopy.

**Quantitative analysis of immunostaining.** Quantitative image analysis of the immunofluorescence for GFAP, Iba-1, and NeuN cells were performed on five cerebral cortical areas of each 4 brain slices taken with the ×40 objective (n = 3 mice per group). Immunofluorescence intensity was calculated using the threshold method and defined as the average number of pixels per slice by the ImageJ software, then divided by the area (mm^2) in the imaged field with the average background subtracted. For quantification of the immunofluorescence double staining, the co-expressed cells in the five regions of the cortex of each 4 brain slices were counted under a microscope (EVOS, AMG) at ×400 magnification (n = 3 mice per group). The results are expressed as an average number of positive cells per unit area (mm^2) of each slice. For the quantitative analysis of inflammatory factors in immunohistochemistry, the investigators who were blinded to the experimental groups randomly collected five high-power field images at ×400 magnification in cerebral cortex areas under a microscope (EVOS, AMG) of each animal (n = 3 mice per group). The cyttoplasmic staining areas that showed light yellow or brownish yellow were selected as positive cells, and the expression of inflammatory factors was quantified by the average count of positive staining cells per animal.

**Statistic methods.** Data are presented as mean ± standard deviation (SD) or standard error of the mean (SEM). For multiple comparisons, one-way analysis of variance (ANOVA) was performed using the SPSS 19 software to assess difference in means among groups and compared with the Sham and TBI groups, analyzed by ANOVA.

**Data availability**

The data that support the findings of this study are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Received: 6 May 2020; Accepted: 17 November 2020; Published online: 07 January 2021

**References**

1. Wei, H. et al. Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes. Chem. Soc. Rev. 42, 6060–6093 (2013).
2. Dydo, P. et al. Artificial metalloenzyme with the kinetics of native enzymes. Science 354, 102–106 (2016).
3. Liang, M. et al. Nanozymes: from new concepts, mechanisms, and standards to applications. Acc. Chem. Res. 52, 2190–2200 (2019).
4. Jiang, D. et al. Nanozyme: new horizons for responsive biomedical applications. Chem. Soc. Rev. 48, 3638–3704 (2019).
5. Huang, Y. et al. Nanozymes: classification, catalytic mechanisms, activity regulation, and applications. Chem. Rev. 119, 4357–4412 (2019).
6. Key, H. M. et al. Abiological catalysis by artificial haem proteins containing noble metals in place of iron. Nature 534, 534–537 (2016).
7. Du, Z. et al. Exosomes for cell-targeted bioorthogonal catalysis. Nat. Catal. 2, 837–838 (2019).
8. Sancho-Albero, M. et al. Cancer-derived exosomes loaded with ultrathin palladium nanosheets for targeted bioorthogonal catalysis. Nat. Catal. 2, 864–872 (2019).
9. Jiang, X. et al. Glutathione-mediated biotransformation in the liver modulates nanoparticle transport. Nat. Nanotechnol. 14, 874–882 (2019).
10. Loynachan, C. N. et al. Renal clearable catalytic gold nanoclusters for in vivo disease monitoring. Nat. Nanotechnol. 14, 843–890 (2019).

11. Li, S. et al. A nanocatalyst with photo-enhanced dual enzyme-like activities for deep pancreatic cancer therapy. Angew. Chem. Int. Ed. 58, 12624–12631 (2019).

12. Fan, K. et al. In vivo guiding nitrogen-doped carbon nanomaterials for tumor catalytic therapy. Nat. Commun. 9, 1440 (2018).

13. Wang, Z. et al. Biomimetic nanoflowers by self-assembly of nanomaterials to in vivo intracellular oxidative damage against hypoxic tumors. Nat. Commun. 9, 3334 (2018).

14. Mu, X. et al. Redox trimetallic nanzyme with neutral environment preference for brain injury. ACS Nano 13, 1870–1884 (2019).

15. Yan, R. et al. Nanomaterial-based scaffold with single-atom catalysis for brain trauma. ACS Nano 13, 11532–11560 (2019).

16. Mu, X. et al. Calixarene nanosheet with ultrahigh reactive nitrogen species selectivity for traumatic brain injury. Nano Lett. 19, 4527–4534 (2019).

17. Lin, Y. et al. Nano-gold as artificial enzymes: hidden talents. Adv. Mater. 26, 4200–4217 (2014).

18. Tao, Y. et al. Bifunctionalized mesoporous silica-supported gold nanoparticles: antimicrobial activity against Gram-positive and Gram-negative bacteria. Angew. Chem. Int. Ed. 58, 132–136 (2019).

19. Xu, Q. et al. Direct electrochemistry of horseradish peroxidase based on biocompatible carboxymethyl chitosan-gold nanoparticle nanocomposite. Biosens. Bioelectron. 22, 768–773 (2006).

20. Wang, J. Y. et al. Hollow PtPdRh nanocubes with enhanced catalytic activities for in vivo clearing of radiation-induced ROS via surface-mediated bond breaking. Small 14, 1703736 (2018).

21. Huang, Y. et al. Self-assembly of multi-nanomaterials to mimic an intracellular antioxidant defense system. Angew. Chem. Int. Ed. 55, 6646–6650 (2016).

22. Gao, Y. et al. Intrinsic peroxidase-like activity of ferromagnetic nanoparticles. Nat. Nanotechnol. 2, 577–583 (2007).

23. Fan, K. et al. Magnetoferritin nanoparticles for targeting and visualizing tumour tissues. Nat. Nanotechnol. 7, 459–464 (2012).

24. Singh, N. et al. A redox modulatory MnO2 nanomaterial with multi-enzyme activity provides efficient cytoprotection to human cells in a Parkinson’s disease model. Angew. Chem. Int. Ed. 56, 14267–14271 (2017).

25. Zhao, S. et al. Enzyme protects the integrity of the blood-brain barrier against experimental cerebral malaria. Nano Lett. 19, 8887–8895 (2019).

26. Zhang, K. et al. Hollow Prussian blue nanomaterials drive neuroprotection against ischemic stroke via attenuating oxidative stress, counteracting inflammation, and suppressing cell apoptosis. Nano Lett. 19, 2812–2823 (2019).

27. Liu, Y. et al. Comprehensive insights into the multi-antioxidative mechanisms of melanin nanomaterials and their application to protect brain from injury in ischemic stroke. J. Am. Chem. Soc. 139, 856–862 (2017).

28. Simon, D. W. et al. The far-reaching scope of neuronal inflammation after traumatic brain injury. Nat. Rev. Neurol. 13, 171–191 (2017).

29. Hao, M. et al. Tumor-selective catalytic nanomedicine by nanocatalyst delivery. Nature. Commun. 8, 357 (2017).

30. Zhang, Z. et al. Molecular imprinting on inorganic nanomaterials for hundred-fold enzyme specificity. J. Am. Chem. Soc. 139, 5412–5419 (2017).

31. Fang, G. et al. Differential Pd-nanocrystal facets demonstrate distinct antibacterial activity against Gram-positive and Gram-negative bacteria. Nat. Commun. 9, 129 (2018).

32. Xu, B. et al. A single-atom nanomaterial for wound disinfection applications. Angew. Chem. Int. Ed. 58, 4911–4916 (2019).

33. Huang, L. et al. Single-atom nanomaterials. Sci. Adv. 5, 5490 (2019).

34. Jiao, L. et al. When nanomaterials meet single-atom catalysis. Angew. Chem. Int. Ed. 132, 2585–2596 (2020).

35. Huo, M. et al. Nanocatalytic tumor therapy by single-atom catalysts. ACS Nano 13, 2643–2653 (2019).

36. Wang, D. et al. Self-assembled single-atom nanomaterials for enhanced photodynamic therapy treatment of tumor. Nat. Commun. 11, 357 (2020).

37. Cao, F. et al. An enzyme-mimicking single-atom catalysts an efficient multiple reactive oxygen and nitrogen species scavenger for sepsis management. Angew. Chem. Int. Ed. 132, 5146–5153 (2020).

38. Imaoka, T. et al. Platinum clusters with precise numbers of atoms for preparative-scale catalysis. Nat. Commun. 8, 688 (2017).

39. Kratki, K. et al. Generation and stabilization of small platinum clusters Pt12ex inside a metal-organic framework. J. Am. Chem. Soc. 141, 13962–13969 (2019).

40. Wang, A. et al. Heterogeneous single-atom catalysis. Nat. Rev. Chem. 2, 65–81 (2018).

41. Zhu, M. et al. Correlating the crystal structure of a thiol-protected Au25 cluster and optical properties. J. Am. Chem. Soc. 130, 5883–5888 (2008).

42. Akola, J. et al. On the structure of thiolate-protected Au25. J. Am. Chem. Soc. 130, 3756–3757 (2008).
Acknowledgements
This work was financially supported by the National Natural Science Foundation of China (Grant Nos. 91859101, 81971744, U1932107, 814717866, and 11804248), the Independent Innovation Foundation of Tianjin University, the Natural Science Foundation of Tianjin (Grant No. 18JCQNJC03200), and the NSF (Grant No. IRES 1826917).

Author contributions
X.-D.Z. conceived and designed the experiments. H.L. contributed to materials synthesis, H.L., S.L., and X.Y. contributed to physical and chemical measurement; Y.L. and K.V. contributed to the simulation of the theoretical calculation; and S.S., Q.X., and K.C. contributed to biological experiment. X.-D.Z., J.X., S.S., H.L., X.M., H.W., W.M., and Y.L. analyzed the data; X.-D.Z., S.S., H.L., and Y.L. prepared the manuscript. All authors discussed the results and commented on the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41467-020-20275-0.

Correspondence and requests for materials should be addressed to X.-D.Z.

Peer review information Nature Communications thanks David Loane, Lizeng Gao, and the other anonymous reviewers for their contribution to the peer review of this work. Peer reviewer reports are available.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2021