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# Protein-mediated wool-ball-like copper sulfide as multifunctional nanozyme for dual fluorescence "turn-on" sensors of cysteine and silver ion

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## ABSTRACT

Developing multifunctional nanozyme based biosensor with convenient approach and high reliability is of vital interests for multiplex detection. Here, wool-ball-like copper sulfide (WBLCS) was obtained facilely using an amphiphilic protein. The acid amino acid residues and the amphiphilic property of protein molecules play cooperative roles in the fabrication of hierarchical nanostructure. Unlike copper sulfide with irregular morphologies, the single component WBLCS acts as a multifunctional nanozyme possessing superior both cysteine oxidase- and peroxidase-mimicking activity. Fascinatingly, the addition of silver ion can significantly enhance the performance of the cascade system at very low fluorescence substrate concentration. Based on this, dual fluorescence "turn on" sensors of cysteine and silver ion with extremely high sensitivity and selectivity are developed. This is the first report to explore multiple fluorescence "turn on" sensing systems based on one single nanozyme. Hence, the present finding has significant implication towards the design of superstructured nanozymes combining different multi-functionalities at the nanoscale for sensing multiplex target molecules sensitively and selectively in practice.

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## 1. Introduction

Many metal ions, as the essential trace elements, have a propensity to engage in the synthesis of enzymes and the metabolism process in the human body.[1-3] Furthermore, heavy metal ions are one of the most harmful environmental pollutants as they are non-biodegradable and easy to accumulate in ecological systems. Amino acids also play significant roles in numerous biological systems, such as protein synthesis, detoxification, and metabolism.[4-6] Disproportionate amounts of metal ions or amino acids lead to the abnormal operation of enzymes and the disordered metabolism process, which will result in many diseases including slow growth, organ damage, edema, and skin diseases. Therefore, developing the multifunctional biosensor with simple operation and high reliability for detection of multiple target analytes, e.g. amino acids and metal ions, will be a new and developing field to be investigated.

As a unique alternative to natural enzymes, studies about nanomaterial artificial enzymes have made great progresses due to their excellent stability, regulatable catalytic activity, low cost, and unique chemical-physical properties.[7-11] The nanozyme-based strategy is currently one of the research focuses in sensing target analytes including heavy metal ions and small organic molecules. Up to now, peroxidase-mimicking nanozymes has received much attention because of their practical applications in biological, medical, environmental and food detection.[12-16] A great number of biosensors based on peroxidase-mimicking nanozymes have been developed since the target analyst can switch the peroxidase-mimicking activity to some extent.[17-21] However, the use of peroxidase-mimicking nanozymes is not easy to be applicable to multiplex detection based on a single nanozyme. Second, most nanozymes based sensors (including metal ion and amino acid sensors) explored to date are due to the optical signal "turn-off" by activity inhibition.[22] Such "turn-off" nanozyme sensors inevitably show the lack of sufficient chemical selectivity and false-positive results. Furthermore, the instability and low

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toxicity of $H_2O_2$ limits the application of peroxidase-mimicking nanozymes in practice. Developing "turn-on" sensing strategies based on nanozymes with sufficient chemical selectivity and sensitivity in the absence of $H_2O_2$ is promising and interesting.

The performances of nanozymes can be modulated facilely by changing their composition, morphology and surface microenvironmental properties.[23-27] Up to now, utilizing noble metal free nanomaterial to construct nanozymes with outstanding and multiply enzyme-mimicking activity via an economically and environmentally sustainable approach remains largely unknown. Recently, copper sulfide based nanozymes has drawn the researcher's interest due to the advantages of simple preparation, low-cost, good stability, good biocompatibility and easy modification.[28-33] However, copper sulfide nanomaterial based nanozymes have been found to exhibit mainly peroxidase activity in earlier studies, which limits their practical application. Thus, it is of great scientific interest and technological importance to study the enzyme-mimicking activities of copper sulfide with different structures.

Proteins have significant advantages in the synthesis of nanomaterial with various superstructures, such as mild reaction conditions and facile operation processes. Furthermore, the modified proteins endow nanomaterial with high specific biological recognition, biocompatibility and diversified functions.[34-36] Here, superstructured wool-ball-like copper sulfide (WBLCS) was obtained facilely using an amphiphilic protein-casein. Casein has a highly content of acid amino acid residues (such as glutamic acid and aspartic acid residues), so the protein can control the crystallization of CuS effectively via the carboxyl groups. Furthermore, similar to amphiphilic block copolymers, caseins consists of blocks with high levels of hydrophobic or hydrophilic amino acid residues.[37,38] Such amphiphilic property contributes to not only the formation of superstructured but also the affinity of substrates. Based on the high-performance of self-cascade reaction of hierarchically nanostructured WBLCS, double fluorescence "turn-on" sensors for selectively and sensitively sensing cysteine and silver

ions are developed (Scheme 1). It is the first report to explore multiple "turn on" sensors based on just a single nanozyme. With the versatile features of multifunction, simplification and low cost, super-structured metal nanozymes will broaden the applicability toward the ultrasensitive and high-selective detection of multiplex target molecules in clinical diagnosis, environmental monitoring, and food quality control.



## 2. Experimental section

### 2.1. Chemicals and materials

Casein was purchased from Sigma (>99%). 3,3′,5,5′-tetramethylbenzidine (TMB), copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$, Sinopharm Chemical Reagent Co., Ltd., 99%), thiourea ($CH_4N_2S$, Sinopharm Chemical Reagent Co., Ltd., 99%), L-Cysteine (Cys., Sigma, >99%), silver nitrate ($AgNO_3$, Sinopharm Chemical Reagent Co., Ltd.). All other reagents were of analytical grade and the water was ultrapure water (18.2 MΩ). The above reagents are used directly. The casein powder was dispersed in water under stirring at 60 °C and stored at 4 °C overnight to allow complete hydration.

### 2.2. Instrumentation and characterization

The activity of the enzymes of the samples was measured by ultraviolet-visible (UV−vis) spectrometer (Shimadzu UV-2501). The steady-state fluorescence experiment was carried out using an RF-5301 fluorescence spectrometer (Shimadzu, Japan) with excitation wavelength of 315 nm and a scanning range of 350-600 nm. X-ray photoelectron spectroscopy (XPS) analyses were performed using an ESCALAB 250Xi spectrometer (Thermo Fisher Co., USA) with an Al X-ray source (1350 eV of photons). Fourier transform infrared (FT-IR) spectra of samples were recorded using FT-IR spectroscopy (Nicolet-740). X-ray diffraction (XRD) was carried out by using an X-ray diffractometer model D8 advance (Bruker) with Cu Kα radiation (λ = 1.5418 Å). The morphology of the samples was observed using a Zeiss Supra 55 field emission

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scanning electron microscope (Carl Zeiss, Germany).

### 2.3. Preparation of wool-ball-like copper sulfide

Using copper sulfate as the copper source and thiourea as the sulfur source, 10 mL of 10 mg/mL casein solution was slowly added to the copper sulfate solution to obtain a casein-copper sulfate mixed solution. After transferring to water bath of 85 °C for 30 min, 25 mL of 0.2 M thiourea solution was added. Finally, the mixed solution reacted at 85 °C for 24 h. The sample was centrifuged, and washed by deionized water and ethanol.

### 2.4. Wool-ball-like copper sulfide as peroxidase mimetics

Add 120 μL of 15 mM 3,3',5,5'-tetramethylbenzidine (TMB) into 2.842 mL of 0.2 M pH 4.0 acetate buffer, and then 8 μL of prepared copper sulfide nanoparticle was added. Finally, 30 μL of 2 M hydrogen peroxide was added. The solution was then transferred for UV−vis scanning after incubating for 15 min.

### 2.5. Determination of cysteine

Add 0.5 mL of 20 mM terephthalic acid solution into 2.9875 mL of 0.2 M pH 5.0 PBS buffer. Then, 12.5 μL of copper sulfide and 0.5 mL of different concentrations of cysteine were added. The mixed solution was reacted at 75 °C for 15 min, and its fluorescence spectrum was measured.

### 2.6. Determination of $Ag^+$

12.5 μL of prepared copper sulfide were added into 0.9675 mL of 0.2 M pH 5.5 PBS buffer, and 20 μL of different concentrations of silver nitrate solution were added. The mixed solution was kept at room temperature for 15 min. Then, 2.0 mL 0.2 M pH 5.5 PBS buffer, 0.5 mL 0.2 mM terephthalic acid solution, 0.5 mL 4 mM cysteine solution were added. The mixture was kept at 70 °C for 15 min, and the fluorescence spectrum was measured.

### 2.7. Detection of cysteine and silver ions in real samples

For Cys sensing, 12.5 μL of copper sulfide, 0.5 mL of 20 mM TA and 40 μL of the serum

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were added into 3.4475 mL pH 5.0 PBS buffer. The mixed solution was reacted at 75 °C for 15 min, and its fluorescence spectrum was measured.

Water samples were collected from Slender West Lake near our university. The collected lake water samples were first filtered through a 0.22 μm membrane. Then, 20 μL of water samples with different silver ion concentration and 12.5 μL of prepared copper sulfide were added into 0.9675 mL pH 5.5 acetate buffer. The mixture was kept at room temperature for 15 min. Then, 2.0 mL 0.2 M pH 5.5 PBS buffer, 0.5 mL 0.2 mM terephthalic acid solution, 0.5 mL 4 mM cysteine solution were added. The mixture was kept at 70 °C for 15 min, and the fluorescence spectrum was measured. The method for determining $Ag^+$ in serum samples is the same.

## 3. Results and discussion

### 3.1. Characterization of wool-ball-like copper sulfide

Fig. 1A is the scanning electron microscope (SEM) image of the copper sulfide obtained in the presence of casein. It can be seen from the figure that the product has a spherical structure with a diameter of about 500 nm. The SEM image of high resolution (Fig. 1B) displays the wool-ball-like superstructure. Fig. 1C is the XRD spectrum of WBLCS. The peaks at 26.53°, 29.24°, 31.75°, 38.73°, 47.98°, 52.54°, and 59.05° correspond to the diffractive surface {100}, {102} , {103}, {105}, {110}, {108}, and {116} of CuS crystal faces, which is consistent with the JCPDS spectrum of the CuS standard (JCPD card No. 06-0464). Fig. 1D is the FT-IR spectra of casein and WBLCS. Compared with the spectrum of casein, the FT-IR spectrum of WBLCS indicates the binding of protein to copper sulfide. The peak at 1645 $cm^{-1}$ for casein indicates an unordered structure of protein. The FT-IR spectrum of WBLCS shows the band at 1630 $cm^{-1}$, indicating the extended chain of protein after binding with CuS. Casein contains aspartic acid and glutamic acid residues with high content, and carboxyl functional groups of the acid amino acid residues can coordinate with cooper ion. This can be confirmed by the significant change

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of the band of $COO^-$ at 1447 and 1398 $cm^{-1}$ contributed by the acid amino acid residues.

To explore further the growth and formation mechanism of the WBLCS superstructure, we examined the intermediate products at different stages with SEM measurements. Fig. 2 is the SEM image of the product obtained at different reaction times. The product obtained at 10 min of the initial reaction is small particles (Fig. 2A). Wire-like structure is formed after a reaction time of 3 h (Fig. 2B). The nanowire is entangled with each other and loose wool-ball-like structure appears as the reaction time extending to 6 h (Fig. 2C). Complete WBLCS superstructures are obtained after the reaction time of 24 h (Fig. 1B). Caseins consist of a highly repetitive sequence of acid amino acid residues. As acidic proteins, caseins have the ability to bind copper ions via carboxylate groups of acid amino acid residues, which is confirmed by the above FTIR results. Furthermore, caseins consisting of blocks with high levels of hydrophobic or hydrophilic amino acid residues, are similar to amphiphilic block copolymers. Thus, van der Waals between nanoparticles, the coordination between protein and copper ion, and the hydrophobic interaction between protein molecules may be the main driving force for the nanoparticle assembly into wire-like structure and further into the wool-ball-like structure. In order to further determine the role of casein in the formation of WBLCS superstructure, we conducted controlled experiments with aspartic acid (Asp), histidine (His) and hydrolyzed casein (HYC) as additives to synthesize copper sulfide nanomaterials. As shown in Fig. S1, copper sulfide synthesized in the presence of His and without additives display irregular structure and copper sulfide synthesized in the presence of Asp and HYC shows spherical-like structure. Asp and His are chosen since they are acid amino acid and basic amino acid containing carboxyl group and amino group, respectively. The result indicates that the carboxyl group plays a crucial role in the formation of the superstructure. HYC is the mixture of small peptides, and some of them bear carboxyl groups. However, different from caseins, the amphiphilic property of these peptides is absent. Thus, HYC is used to confirm the amphiphilic

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role of protein in the formation of the WBLCS superstructure.

### 3.2. Peroxidase activity of wool-ball-like copper sulfide

3,3',5,5'-Tetramethylbenzidine (TMB) as a common substrate was chosen to investigate the peroxidase-like activity of WBLCS. Fig. 3A shows the UV-Vis spectra of different systems. It can be seen from the figure that the color of the system changes from colorless to blue when the WBLCS is mixed with $H_2O_2$ and TMB, corresponding to an absorption peak at 650 nm. At the same time, the solution is nearly colorless for the TMB-$H_2O_2$ and TMB-WBLCS system, and there is no absorption peak at 650 nm in the UV-Vis absorption spectrum, indicating that WBLCS exhibits peroxidase-like activity. Compared with $Cu^{2+}$ and CuS nanomaterials synthesized without additives (curve c and d in Fig. 3A), the peroxidase-like activity of WBLCS is significantly enhanced, which indicates that outstanding peroxidase-like activity depends much on the superstructure of WBLCS. To confirm the role of protein in enhancing the peroxidase-like activity of the hybrid nanozyme, the catalytic activity of the mixtures of $Cu^{2+}$ + protein and CuS + protein has been added in Fig. S2. As shown, the peroxidase-like activity of $Cu^{2+}$ + protein and CuS + protein is higher than $Cu^{2+}$ and CuS, especially for the latter, which confirms the role of protein in determining the peroxidase-like activity of copper nanozyme. Even so, the mixture of CuS and protein displays much lower enzyme-like activity than WBLCS. This demonstrates the peroxidase-like of WBLCS depends much on the cooperative effect of the wool-ball-like structure of CuS and proteins. We have emphasized this in the revised manuscript.

To confirm the crucial role of such WBLCS superstructure, the peroxidase-like activity of the above copper sulfide prepared at different conditions were studied. Fig. 3B shows the peroxidase-like activity of CuS obtained in the presence of casein at different reaction time. As the reaction progresses, the peroxidase-like activity is gradually enhanced. Also, the activity of CuS with irregular structure or spherical-like structure is very low at the same experimental

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conditions (inset in Fig. 3B). Therefore, the WBLCS superstructure really matters much in determining the enzyme-like activity of copper sulfide.

Similar to natural enzymes, temperature, pH, and substrate concentration all affect the catalytic activity of WBLCS. As shown in Fig. S3, the optimal pH and temperature are pH 4.0 and 25 ℃, respectively. The peroxidase-like activity of WBLCS was further evaluated by determining the kinetic parameters using the initial rate method. According to Lineweaver–Burk plot, Michaelis–Menten constant ($K_m$) and maximum initial velocity ($V_{max}$) were obtained (Fig. S4). It can be seen from Table S1, $K_m$ values for TMB and $H_2O_2$ are 0.435 and 0.04 mM, so WBLCS have an extremely strong affinity for TMB and $H_2O_2$. Significantly, metallic oxide based nanozymes usually shows a weak affinity towards $H_2O_2$, but the present WBLCS shows a much stronger affinity towards $H_2O_2$ than HRP. Moreover, the maximum reaction rates of both TMB and $H_2O_2$ are greater than HRP. The affinity of substrates towards nanozymes depends much on their composition, morphology and surface microenvironmental properties. As shown in Table S1, CuS based nanozyme for TMB and $H_2O_2$ varies much. Considering the $K_m$ values of both TMB and $H_2O_2$, the present WBLCS displays high affinity towards the two substrates. As discussed above, casein is an amphiphilic protein containing a high content of acid amino acid residues. Therefore, both the electrostatic attraction and hydrophobic interaction contributes to the high affinity of TMB towards WBLCS. In addition, different from the irregular shaped CuS obtained in the absence of protein, the cooperative effect of the wool-ball-like structure and protein is further propitious to the high affinity for substrates. Thus, the wool-ball-like superstructure of copper sulfide has significant influence on the enzyme-like activity of nanozymes, and the fabrication of superstructures will provide more chances to improve the performance of nanozymes.

### 3.3. Detection of cysteine based on self-cascade reaction of WBLCS

Based on the above peroxidase-like activity of WBLCS with TMB as the substrate, one

fluorescence probe terephthalic acid (TA) was used. As shown in Fig. S5, WBLCS catalyze the oxidation reaction of TA and $H_2O_2$, and a highly blue fluorescent hydroxyterephthalate (TAOH) is obtained. The controlled results show that TAOH is not produced in WBLCS-TA system due to the absence of hydrogen peroxide. Fascinatingly, highly fluorescent TAOH was obtained in WBLCS-TA-Cys system without the addition of $H_2O_2$ (Fig. 4A). This demonstrates that WBLCS displays cysteine oxidase (CTO)-mimicking activity, which can catalyze the oxidation reaction of Cys and $O_2$ to obtain cystine and $H_2O_2$. Some controlled experiments were conducted to confirm this and the results are shown in Fig. 4B. The fluorescent intensity of WBLCS-TA-Cys system decreases significantly after the saturation of nitrogen, demonstrating the participation of dissolved $O_2$ in the reaction. The formation of hydroxyl radicals from $H_2O_2$ is further confirmed by introducing a hydroxyl radical scavenger, EDTA, into the WBLCS-TA-Cys system. Expectedly, the fluorescence intensity of the system decreases significantly after the addition of EDTA (curve c in Fig. 4B).

The results here confirm that WBLCS processes effective both peroxidase-like and CTO-like activity. Thus, a self-organized cascade is developed based on the single component WBLCS, which can be used to detect cysteine conveniently (Scheme 1a). WBLCS catalyzed the oxidation of Cys to cystine by $O_2$ based on the CTO-like activity of WBLCS. At the same time, produced $H_2O_2$ is confined in the wool-ball-like structure to react with TA to form the highly fluorescent TAOH based on the peroxidase-like activity of WBLCS.

In order to optimize the experimental conditions, the effects of reaction temperature, solution pH, TA concentration and reaction time on the self-cascade ration of WBLCS were studied. As shown in Fig. S6, the optimal reaction temperature of 75 °C, pH 5.0, the TA concentration of 2.5 mM, and the reaction time of 15 min were used in the following experiments.

Thiol-containing amino acid, cysteine plays crucial roles in various physiological processes. Therefore, quantitative analysis of cysteine is essential. The determination of Cys was measured

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under optimal conditions according to the fluorescence intensity changes. Fig. 5A is the fluorescence spectra of WBLCS-TA system at different cysteine concentrations. The results show a good linear relationship between fluorescence intensity and cysteine concentration in the range of 0.03 to 125 μM ($R^2$ = 0.995) (Fig. 5B). The limit of detection (LOD) of cysteine (S/N=3) was 1 nM. The analytical performance of the present Cys sensor was compared with that of other sensors in earlier studies (Table S2). The sensor has a wider linear range, high sensitivity and low LOD, exhibiting a good application prospect.

Specificity is a crucial index for the performance of Cys sensor, so the selectivity of detection towards other biomolecules was further studied. Fig. S7 shows the fluorescence intensity of WBLCS-TA systems with the addition of different biomolecules. As shown in the figure, the addition of other biomolecules has no significant effect on the fluorescence intensity of the system. This proves that the WBLCS-TA based "turn on" fluorescence sensor has good selectivity for Cys.

To demonstrate the reliability of the present method, the fluorescence detection of Cys in human serum was investigated. The human serum was diluted to 100-fold in the final sensing system. As shown in Table S3, 230 μM Cys was found in the volunteer's blood sample, which is consistent with the Cys level of healthy human. Then, the standard addition method was used, and the recovery value is from 98.3% to 104.0%, indicating that the present fluorescence method can be used to detect Cys in serum samples.

### 3.4. Detection of silver ion based on enhancing self- cascade reaction of WBLCS

Amazingly, the fluorescence intensity was significantly enhanced after the addition of trace amounts of $Ag^+$ to WBLCS-TA-Cys systems at very low TA concentration (0.025 mM) (Fig. S8). The controlled experimental result indicates that the addition of individual silver ion does not lead to the enhancement of the fluorescence intensity of TA. To obtain optimal experiment conditions for sensing $Ag^+$, the effects of temperature, pH, TA concentration and Cys

concentration on the catalytic enhancement of WBLCS-TA-Cys-$Ag^+$ system was conducted (Fig. S9). It can be seen from the figure that the optimal temperature, pH, TA concentration, Cys concentrations were 70 °C, pH 5.5, 0.025 mM, and 0.5 mM, respectively.

Silver ion, as one of the most dangerous metal pollutants, has an adverse effect on the environment and is easy to accumulate in the human body, resulting in serious harm to human health. Under optimal conditions, the fluorescence sensing $Ag^+$ was measured. The fluorescence intensity of WBLCS-TA-Cys system increases linearly with the increase of $Ag^+$ concentration in the range of 50 nM to 75 μM (Fig. 6). The correlation coefficient is 0.993, and the detection limit of silver ions can reach 5 nM (S/N=3). The comparison for silver ion sensing using different methods is shown in Table S4. Considering the convenient process, the wide linear range and low LOD, the performance of the present fluorescence turn on method based on WBLCS has well potential for practical application.

In order to further investigate the selectivity of the WBLCS system for the detection of silver ions, various metal ions are used for comparison. The metal ions include $Mn^{2+}$, $Mg^{2+}$, $Pb^{2+}$, $Cr^{3+}$, $Ni^{2+}$, $Cd^{2+}$, $Ca^{2+}$, $Zn^{2+}$, $Al^{3+}$, $Co^{2+}$, $K^+$, $Fe^{3+}$, $Fe^{2+}$ and $Hg^{2+}$ (Fig. S10). The silver ion concentration was 0.075 mM, and the other metal ion concentration was 0.5 mM. Other metal ions cannot turn on the fluorescence of WBLCS-TA-Cys system, indicating the high selectivity towards silver ion.

The present method of WBLCS sensor was used to measure $Ag^+$ concentration in serum and Slender West Lake water samples, demonstrating the feasibility of the sensor based on WBLCS. ICP-OES measurement indicates there is no detectable $Ag^+$ in the real sample. Thus, the standard addition method was selected to sensing silver ion. As shown in Table S5, the recoveries are from 97.7% to 103.2%, which indicates that the sensor can be used for accurate detection of $Ag^+$ in real samples. Notably, as shown in Fig. 5B, the fluorescence intensity of the system has almost reached the plateau in the presence of 0.5 mM Cys. Furthermore, the serum

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has been diluted before use, so the further addition of serum containing Cys will not lead to an obvious change in the fluorescence intensity. Hence, the fluorescence turn on sensor based on WBLCS-TA-Cys system for silver ion in serum is reasonable.

In order to further explore the effect of $Ag^+$ on the enzyme-mimicking activity of WBLCS, the effect of silver ions on the structure of WBLCS were conducted by using SEM, FTIR, XRD and XPS measurement. As shown in Fig. 7A, the SEM image indicated the structure of the WBLCS did not change significantly. The binding energies of Ag $3d_{5/2}$ and Ag $3d_{3/2}$ at 367.9 eV and 373.9 eV in the XPS fine spectrum (Fig. 7B) confirm the binding of silver ions to WBLCS surfaces. Fig. 7C is the FTIR spectra of the WBLCS before and after the addition of $Ag^+$. The absorption peak of $COO^-$ at 1385 $cm^{-1}$ is obviously changed, indicating that the acid amino acid residues in protein play a crucial role in the binding of silver ion to WBLCS. In addition, the strong S–Ag interaction may also lead to the binding of $Ag^+$ to WBLCS. Fig. 7D shows the XRD spectrum of WBLCS after adding $Ag^+$. Comparing with the XRD patterns of WBLCS in Fig. 1C, the XRD spectrum of WBLCS-$Ag^+$ remains almost unchanged, so silver sulfide is not formed in the system. Nanozyme surface plays a critical role in modulating substrate/product adsorption and electron transfer, thus influencing the enzyme-like activity of nanozymes. Obviously, the binding of silver ions to the surface of the WBLCS accelerates the electron transfer to $H_2O_2$ to produce •OH, exhibiting enhancement effects on the self-cascade performance of WBLCS (Scheme 1b).

## 4. Conclusion

In this work, wool-ball-like copper sulfide (WBLCS) was obtained facilely in the presence of amphiphilic protein-casein. WBLCS nanozyme exhibits outstanding peroxidase-/cysteine oxidase-like activity due to its superstructure. The corresponding self-cascade based fluorescence "turn on" sensor is developed for detection of cysteine without $H_2O_2$ and the limit of detection is 1 nM. More interestingly, the performance of self-cascade reaction is enhanced

significantly in the presence of silver ions at very low TA concentration. By virtue of this specific response, another fluorescent "turn-on" sensor is developed, which allows the detection of silver ion in the range of 0.05-75 μM with a detection limit of 5 nM. Furthermore, we demonstrated the usability of the present approach for the detection of cysteine and silver ions in serum and water samples.



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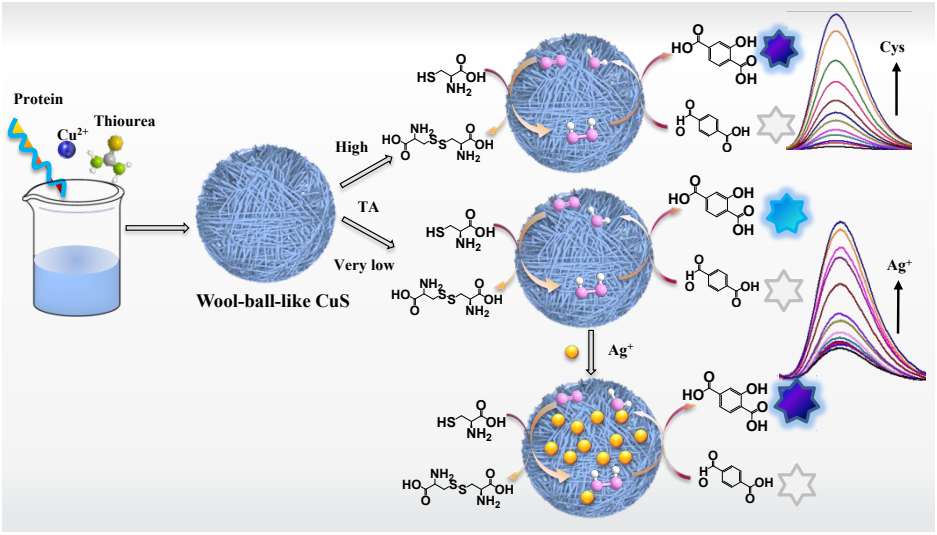


Scheme 1. Schematic illustration of protein-mediated wool-ball-like copper sulfide as multifunctional nanozyme for dual fluorescence "turn on" sensors of cysteine and silver ion.

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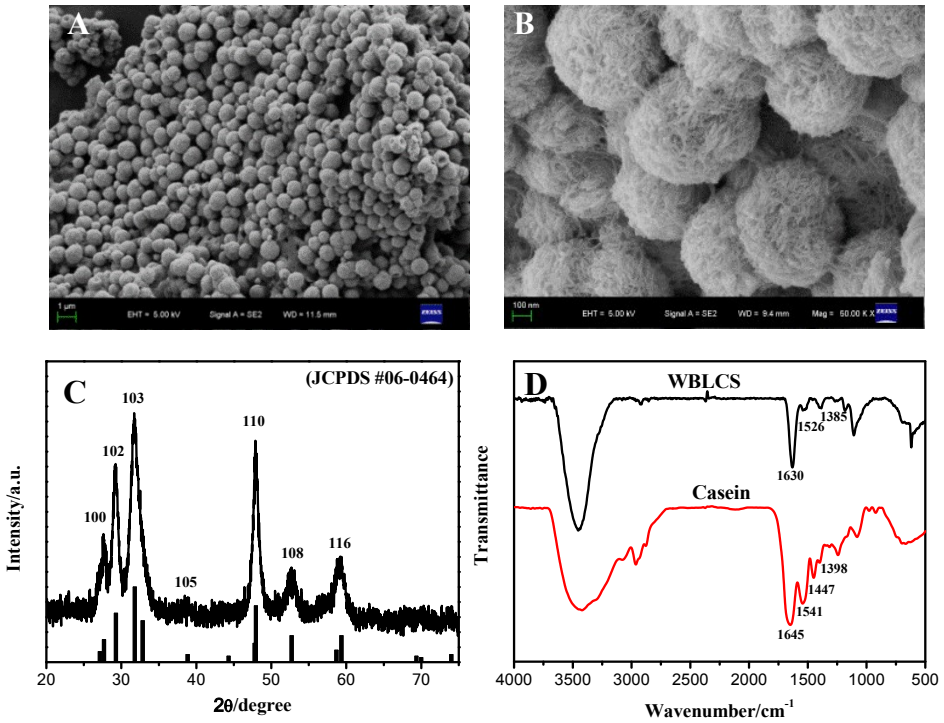


Fig. 1. SEM image (A, B), XRD pattern (C) and FT-IR spectra (D) of WBLCS prepared in the presence of casein.

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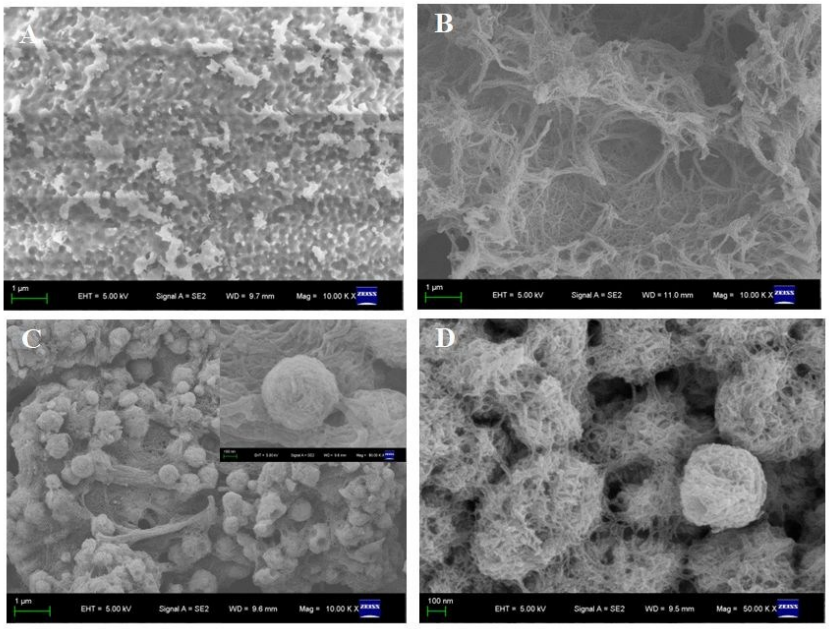


Fig. 2. SEM images of copper sulfide prepared in the presence of casein at different reaction times: 10 min (A), 3 h (B), 6 h (C), and 12 h (D).

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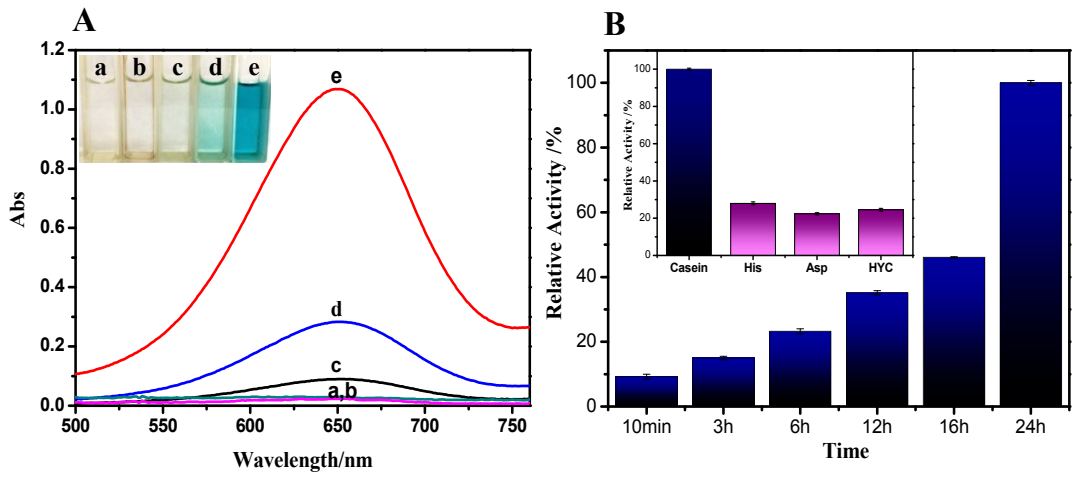


Fig. 3. (A) UV-vis spectra of different systems (a: TMB + $H_2O_2$, b: TMB + WBLCS, c: TMB + $Cu^{2+}$ + $H_2O_2$, d: TMB + CuS + $H_2O_2$, e: TMB + WBLCS + $H_2O_2$). (B) The peroxidase-like activity of copper sulfide obtained at different reaction times in the presence of protein. Inset being the peroxidase-like activity of copper sulfide obtained in the presence of different additives.

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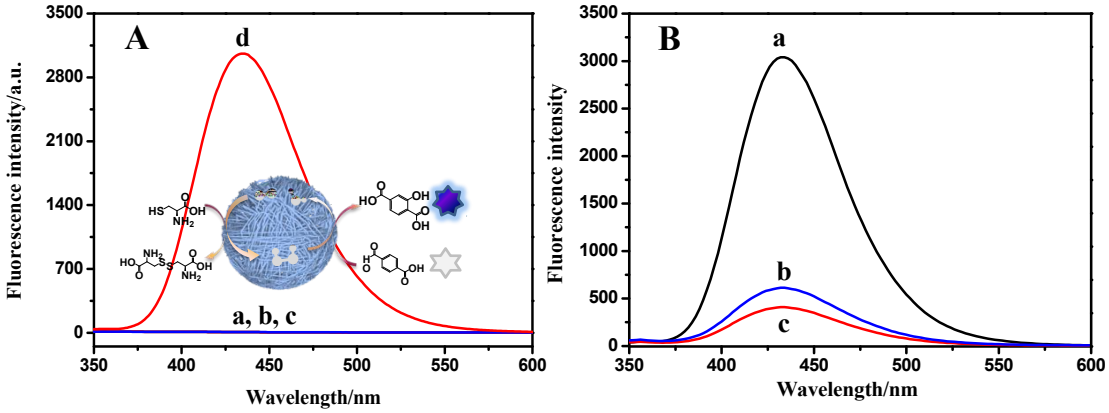


Fig. 4. (A) Fluorescence spectra of different systems (a: TA, b: WBLCS + TA, c: TA + Cys, d: WBLCS + TA + Cys). (B) Fluorescence spectrum of different systems (a: WBLCS + TA + Cys, b: WBLCS + TA + Cys ($N_2$), c: WBLCS + TA + Cys + EDTA (1mM))

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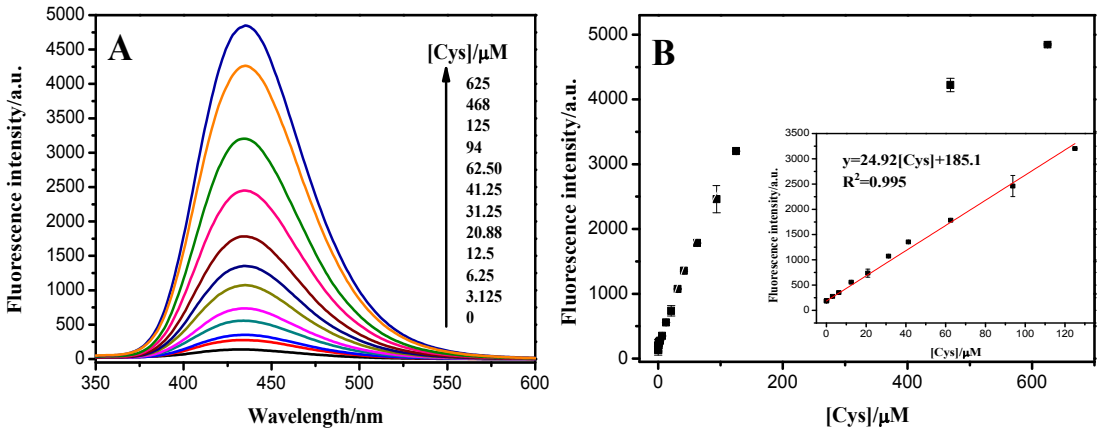


Fig. 5. (A) Fluorescence spectra of WBLCS-TA system in the presence of cysteine with different concentrations. (B) Relationship between the fluorescence intensity and cysteine concentration.

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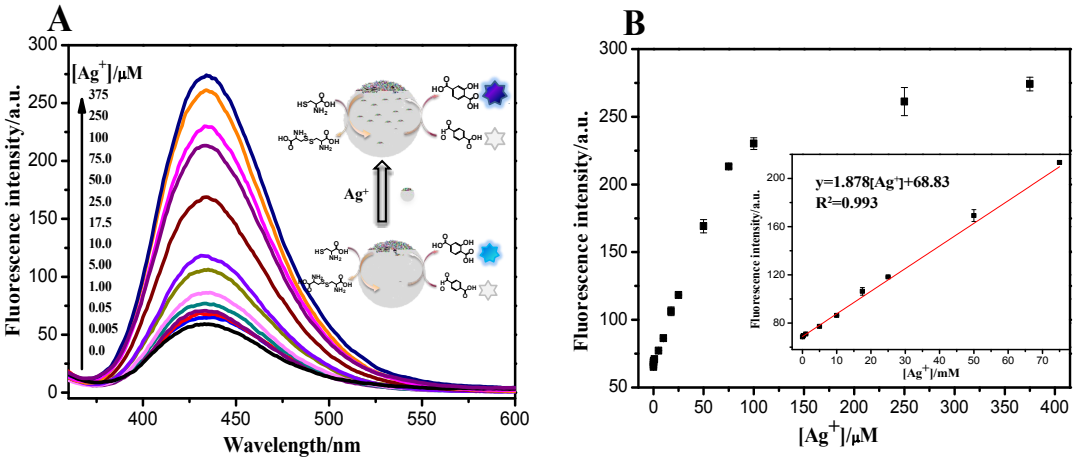


Fig. 6. (A) Fluorescence spectra of WBLCS-Cys-TA system in the presence of $Ag^+$ with different concentrations, [Cys] = 0.5 mM. (B) Relationship between the fluorescence intensity of WBLCS-Cys-TA system and $Ag^+$ concentration.

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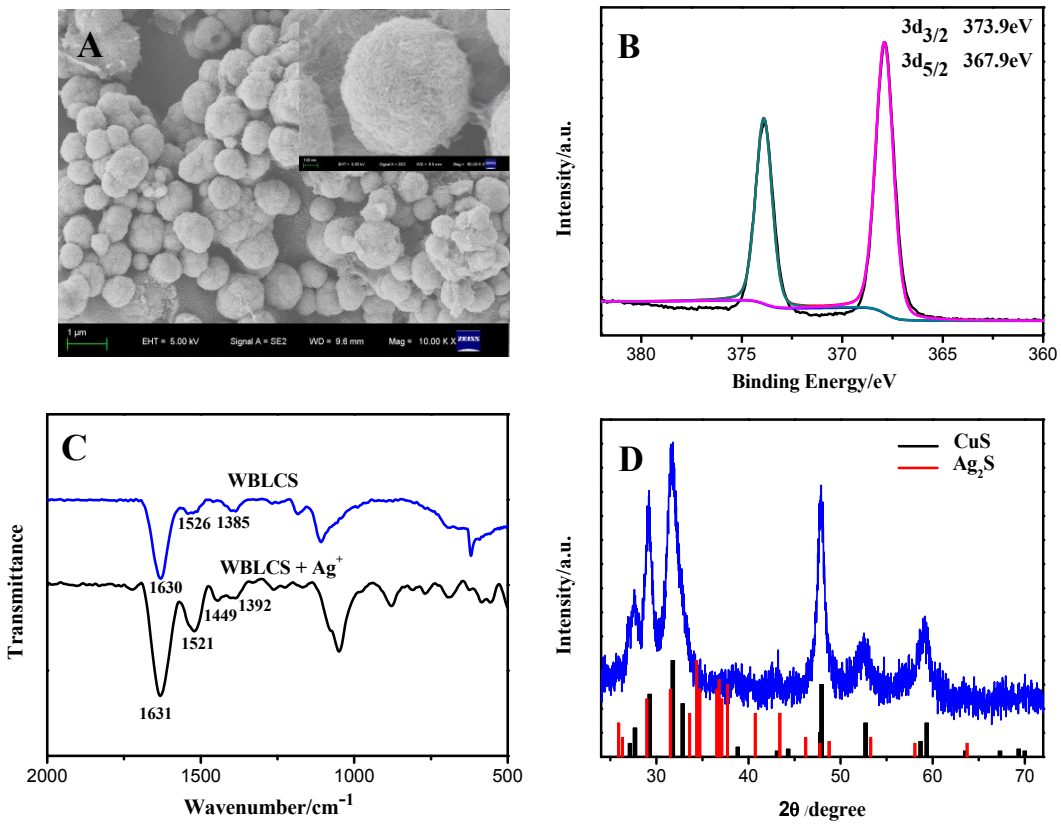


Fig. 7. SEM image (A), XPS spectrum of Ag 3d (B), FTIR spectrum (C) and XRD spectrum (D) of WBLCS-$Ag^{+}$.

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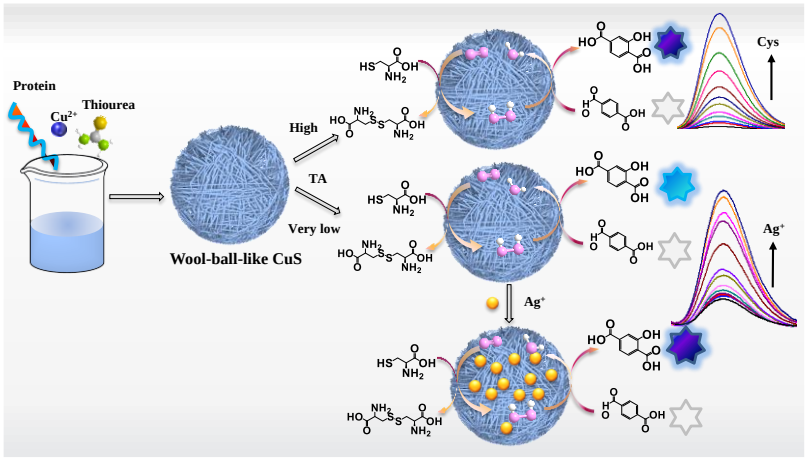


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