

Cobalt Single-Atom Oxidase Mimics for Glucose Oxidase-Free Cascade Colorimetric Detection of α -Glucosidase Activity

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Abstract: The precise evaluation of α -glucosidase (α -Glu) activity is important for diabetes-related diagnosis and drug screening. However, conventional nanozyme-based α -Glu assays rely on multienzyme cascade systems involving glucose oxidase (GOx), which increase assay complexity and introduce instability associated with hydrogen peroxide (H_2O_2). Herein, a GOx-free cascade colorimetric platform based on an atomically dispersed cobalt nanozyme (Co-NC) was established for α -Glu activity detection and inhibitor evaluation. The Co-NC catalyst exhibited intrinsic oxidase-like activity and directly activated dissolved oxygen to trigger the oxidative coupling of hydroquinone (HQ) with 4-aminoantipyrine (4-AAP). By coupling the α -Glu-mediated hydrolysis of α -Arbutin with the Co-NC-mediated chromogenic reaction, the enzymatic event was converted into a measurable optical response at 507 nm without the involvement of GOx and the unstable intermediate H_2O_2 . The biosensor achieved sensitive quantification of α -Glu with a limit of detection of 0.19 mU/mL and satisfactory performance in serum samples. In addition, the method was successfully applied to the evaluation of three commercial α -Glu inhibitors. The proposed Co-NC-based cascade strategy provides a practical and reliable tool for α -Glu monitoring and the proof-of-concept evaluation of representative α -Glu inhibitors.

Keywords: cobalt single-atom nanozyme; oxidase mimic; α -glucosidase; hydroquinone; cascade colorimetric assay; inhibitor evaluation

1. Introduction

α -Glucosidase (α -Glu), a membrane-bound enzyme mainly located in the epithelial cells of the small intestine, plays an important role in carbohydrate metabolism by hydrolyzing the terminal hydrolysis of dietary carbohydrates into absorbable monosaccharides [1,2]. Abnormally elevated α -Glu activity is closely associated with metabolic disorders, particularly type 2 diabetes mellitus, and α -Glu inhibition is an effective strategy for controlling postprandial hyperglycemia [3,4]. Therefore, the determination of α -Glu activity and evaluation of α -Glu inhibitors (AGIs) are important for diabetes management and drug discovery [5–7]. In addition, α -Glu activity has been reported to correlate positively with sperm density and motility [8]. These highlight the need for accurate determination of α -Glu activity and efficient screening of its inhibitors. Although conventional analytical methods, including electrochemical analysis [2], high-performance liquid chromatography [9], and surface-enhanced Raman spectroscopy [8], generally provide good sensitivity, their reliance on costly instrumentation and/or laborious sample preparation may limit their application in rapid and cost-effective analysis.



To overcome these limitations, optical sensing methods have attracted considerable attention because of their operational simplicity and rapid response [3,10–14]. Among them, nanozyme-based optical assays are particularly attractive due to their signal amplification capability, good stability, and relatively low cost [15–17]. Nanozymes are nanomaterials with enzyme-like catalytic activity, whose catalytic properties can be rationally tailored through structural and compositional engineering [18–21]. Coupling enzymatic recognition with nanozyme-mediated catalysis further enables the conversion of enzymatic events into measurable optical signals, providing a useful basis for cascade sensing [22]. Current nanozyme-based α -Glu assays mainly follow two routes using substrates such as 2-O- α -D-glucopyranosyl-L-ascorbic acid (AAG), α -Arbutin, or maltose. In the first route, enzymatic hydrolysis products, including ascorbic acid (AA) [10,23,24] and hydroquinone (HQ) [11,25,26], act as reductants to bleach chromogenic products or inhibit nanozyme activity. However, this turn-off sensing mode usually suffers from relatively high background signals. The second route relies on a multienzyme cascade composed of α -Glu, glucose oxidase (GOx), and peroxidase-like nanozymes [12,27,28]. Such systems involve multiple natural enzymes and hydrogen peroxide (H_2O_2)-dependent signal generation, which can increase operational complexity and compromise assay robustness [29]. Therefore, it is highly desirable to develop a GOx-free sensing strategy that directly couples α -Glu-mediated hydrolysis with oxidase-mimicking catalysis.

Among various nanozymes, cobalt-based nanozymes are of particular interest because they possess intrinsic oxidase-like activity and can directly activate dissolved oxygen for substrate oxidation [30,31]. This feature makes them well suited for constructing cascade systems without the participation of auxiliary oxidases. In particular, cobalt single-atom nanozymes have emerged as a promising subclass owing to their nearly complete metal atom utilization and well-defined coordination structures [32–34]. In these materials, the isolated cobalt centers are anchored within a nitrogen-coordinated carbon framework, giving rise to uniform catalytic sites and tunable electronic environments. Such structural characteristics enable effective substrate activation, efficient electron transfer, and good catalytic stability.

Based on the above considerations, a GOx-free cascade colorimetric platform was designed for α -Glu activity detection by directly integrating substrate hydrolysis with oxidase-like nanozyme catalysis. In this system, α -Glu selectively catalyzed the hydrolysis of α -Arbutin to generate HQ, which then participated in the Co-NC-catalyzed oxidative coupling reaction with 4-aminoantipyrine (4-AAP) to generate a pink optical signal. Distinct from conventional multienzyme cascade assays, this design avoided the use of GOx and the dependence on H_2O_2 , which simplified the assay procedure and improves analytical robustness. Based on this design, the proposed platform was expected to provide a useful approach for α -Glu determination and AGIs evaluation.

2. Materials and Methods

2.1. Materials and Reagents

$Co(NO_3)_2 \cdot 6H_2O$, $Zn(NO_3)_2 \cdot 6H_2O$, 2-methylimidazole (2-MeIm), HQ, phenol, 4-AAP, catalase (CAT), superoxide dismutase (SOD), bovine serum albumin (BSA), Trypsin, urate oxidase (UAO), glutathione reductase (GR), NaCl, KCl, $CaCl_2$ and Na_2SO_4 were obtained from Sigma-Aldrich (St. Louis, MO, USA). α -Glu, fetal bovine serum and α -Arbutin were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). 3,3',5,5'-tetramethylbenzidine (TMB), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid ammonium salt) (ABTS), o-phenylenediamine (OPD), isopropanol (IPA), tryptophan (Try), $MgCl_2 \cdot 6H_2O$, acarbose, voglibose, miglitol, Pepsin, Lysozyme, and Pancreatin were obtained from Shanghai Aladdin Bio-Chem Technology Co., Ltd. (Shanghai, China). Dimethyl pyridine N-oxide (DMPO) was purchased from Bide Pharmatech Co., Ltd. (Shanghai, China). 2,2,6,6-tetramethylpiperidinyl-1-oxide (TEMP) was obtained from Macklin Biochemical Technology Co., Ltd. (Shanghai, China). Na_2CO_3 was purchased from Shanghai Hushi Chemical Co., Ltd. (Shanghai, China).

2.2. Material Characterization

Transmission electron microscopy (TEM) images were acquired on a JEM-2100 Plus electron microscope (JEOL, Tokyo, Japan). High-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) images were obtained on an FEI Themis Z microscope equipped with a probe corrector and a monochromator at 200 kV. Aberration-corrected high-angle annular dark-field scanning transmission electron microscopy (AC HAADF-STEM) image was taken by a JEM-ARM200F spherical aberration-corrected transmission electron microscope from Nippon Electron Co. (Tokyo, Japan). Powder X-ray diffraction (XRD) patterns were collected using an Ultima IV diffractometer (Rigaku, Japan). X-ray photoelectron spectroscopy (XPS) measurements were conducted on an ESCALAB 250XI spectrometer (Thermo, Waltham, MA, USA). UV-vis absorption spectra were recorded on an UV-8000 spectrophotometer (Shanghai, China). Electron paramagnetic

resonance (EPR) spectra were measured on a Bruker ESR5000 spectrometer (Ettlingen, Baden-Württemberg, Germany, Germany). Fourier transform infrared spectroscopy (FT-IR) spectra were recorded on a Nicolet iS50 spectrometer (ThermoFisher Scientific, Waltham, MA, USA).

2.3. Synthesis of Co-NC

Typically, $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (3.80 g) and $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (1.13 g) were dispersed in 40 mL of methanol and ultrasonicated for 10 min to obtain a homogeneous purple solution. Separately, 2-MeIM (1.314 g) was dissolved in 20 mL of methanol under sonication for 10 min. The resulting 2-MeIM solution was then quickly added to the metal precursor solution, followed by stirring at room temperature for 24 h. The precipitate was collected by centrifugation, washed several times with methanol, and vacuum-dried overnight at 60 °C to yield Co@ZIF-8. Subsequently, the obtained precursor was pyrolyzed at 900 °C for 3 h under a flowing nitrogen atmosphere with a heating rate of 5 °C·min⁻¹ to produce Co-NC. For comparison, NC was prepared under the same conditions, except that $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ was omitted.

2.4. Oxidase-Like Activity of Co-NC

To evaluate the oxidase-like activity of Co-NC, 10 µL of Co-NC aqueous dispersion (0.2 mg/mL), 40 µL of TMB solution (10 mM), and 950 µL of acetate buffer (0.2 M, pH 4.0) were mixed in a 1.5 mL centrifuge tube. After incubation at 40 °C for 10 min, the UV-vis absorption spectra were recorded.

Steady-state kinetic analysis for TMB oxidation was performed at room temperature using different TMB concentrations. The obtained data were fitted to the Michaelis-Menten equation to determine the apparent kinetic parameters, including K_m and V_{max} [35]:

$$V = \frac{V_{\max} [S]}{K_m + [S]} \quad (1)$$

where V is the initial reaction velocity, $[S]$ is the substrate concentration, K_m is the Michaelis constant, and V_{max} is maximal reaction velocity.

2.5. EPR Experiment

For the detection of hydroxyl radicals ($\cdot\text{OH}$), 100 µL of Co-NC aqueous dispersion (0.1 mg/mL) was added to 800 µL of acetate buffer (0.2 M, pH 4.0), followed by the addition of 100 µL of DMPO (500 mM). After incubation for 10 min, the resulting solution was extracted by a quartz capillary tube and placed in a glass tube for EPR measurement. The detection of superoxide radicals ($\text{O}_2^{\cdot-}$) was carried out in the same manner, except that methanol was used instead of the aqueous medium. For singlet oxygen ($^1\text{O}_2$) detection, TEMP was used as the spin-trapping agent in an aqueous system.

2.6. Colorimetric Detection of HQ

Typically, 100 µL of HQ solution at different concentrations was added to a mixture containing 830 µL of acetate buffer (0.2 M, pH 4.0), 10 µL of Co-NC aqueous dispersion (0.2 mg/mL), and 60 µL of 4-AAP solution (10 mM). After incubation at 35 °C for 30 min, the UV-vis absorption spectra were recorded.

2.7. Colorimetric Detection of α -Glu

Typically, 100 µL of α -Glu solution with different activities was pre-incubated with 35 µL of α -Arbutin (10 mM) and 20 µL of PBS buffer (0.2 M, pH 7.4) at 37 °C for 30 min to allow enzymatic hydrolysis. Then, 775 µL of acetate buffer (0.2 M, pH 4.0), 10 µL of Co-NC dispersion (0.2 mg/mL), and 60 µL of 4-AAP solution (10 mM) were added to initiate the colorimetric reaction. After a further incubation at 37 °C for 30 min, the UV-vis absorption spectra were recorded.

2.8. α -Glu Inhibition Study

AGI evaluation was carried out using the same procedure as that for the α -Glu assay, except that α -Glu was pre-incubated with different concentrations of acarbose, voglibose, or miglitol at 37 °C for 15 min before the addition of α -Arbutin.

3. Results and Discussion

3.1. Synthesis and Characterization of Co-NC

The synthesis route of Co-NC is illustrated in Figure 1a. Co-NC was obtained through pyrolysis of the Co@ZIF-8 precursor, and its morphology was first examined by TEM. As shown in Figures 1b and S1, Co-NC largely retained the rhombic dodecahedral morphology inherited from the precursor, with an average particle size of about 200 nm. Figure S2 further shows that both pristine ZIF-8 and Co@ZIF-8 displayed well-defined polyhedral structures, indicating that cobalt incorporation did not noticeably affect the formation of the ZIF framework. In contrast, the Co-free control underwent partial structural collapse after pyrolysis (Figure S3). High-resolution TEM (HRTEM) image showed no discernible metal nanoparticles, nanoclusters, or lattice fringes associated with cobalt species, indicating the absence of crystalline cobalt aggregates and the amorphous nature of the carbon matrix (Figure 1c). AC HAADF-STEM image showed numerous bright dots uniformly dispersed over the carbon framework, which are attributed to isolated cobalt atoms (Figure 1d). In addition, EDS elemental mapping illustrates the homogeneous distribution of Co, C, and N elements throughout the entire particle (Figure 1e).

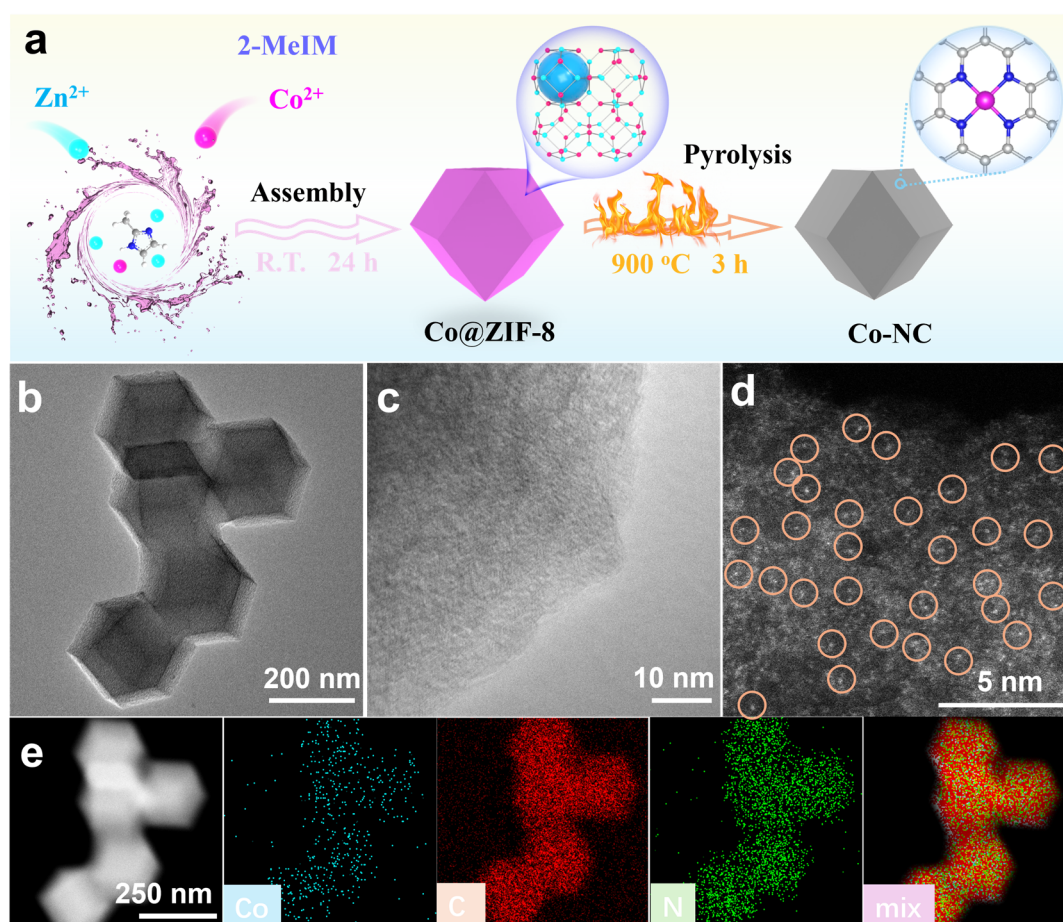


Figure 1. (a) Schematic illustration of the synthesis procedure for Co-NC. (b) TEM, (c) HRTEM, and (d) AC HAADF-STEM images of Co-NC. (e) HAADF-STEM image and corresponding EDS elemental maps of Co-NC.

The crystalline structures of NC and Co-NC were examined by XRD. As shown in Figure 2a, both samples exhibit two broad diffraction peaks at approximately 24.2° and 42.4°, which correspond to the (002) and (101) planes of partially graphitized carbon, respectively [33,36]. Notably, the Co-NC pattern presents no diffraction peaks related to metallic Co or cobalt oxides [37]. This suggests that cobalt species are highly dispersed without forming crystalline aggregates, a finding consistent with the HRTEM results. In addition, FT-IR spectroscopy was used to analyze the surface functional groups. The FT-IR spectra of both materials (Figure 2b) exhibit a broad band at approximately 3456 cm⁻¹ assigned to the O-H stretching vibration, as well as peaks at 1617 and 1382 cm⁻¹ originating from C=O and N-H vibrations, respectively [38]. The existence of these hydrophilic groups likely facilitates the water dispersibility of the catalysts.

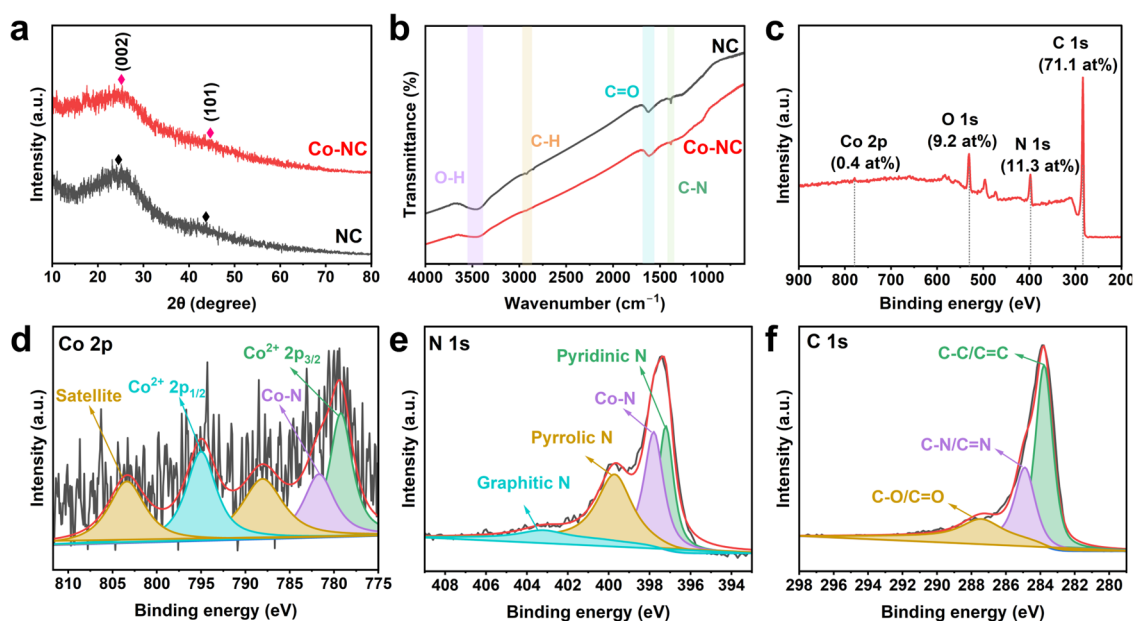


Figure 2. (a) XRD patterns of NC and Co-NC. (b) FT-IR spectra of NC and Co-NC. (c) XPS survey spectrum of Co-NC. XPS spectra of (d) Co 2p, (e) N 1s and (f) C 1s for Co-NC.

XPS was further employed to analyze the surface elemental composition and chemical states of Co-NC. The survey spectrum (Figure 2c) confirms the presence of Co (779.77 eV), N (397.48 eV), C (283.87 eV), and O (531.33 eV), indicating that these elements constitute the main framework of the material. In the high-resolution Co 2p spectrum (Figure 2d), the peaks located at 779.24 and 795.01 eV are assigned to Co 2p_{3/2} and Co 2p_{1/2}, respectively, together with their satellite peaks [36], suggesting that cobalt mainly exists in Co²⁺ state. The high-resolution N 1s spectrum (Figure 2e) can be deconvoluted into components attributed to pyridinic N (397.21 eV), Co-N (397.8 eV), pyrrolic N (399.72 eV), and graphitic N (403.23 eV) [39]. Among these species, the Co-N component provides direct evidence for the coordination of single Co atoms with the N-doped carbon matrix, forming the active Co-N_x moieties. In addition, the C 1s spectrum (Figure 2f) can be resolved into three peaks associated with C=C/C-C (283.78 eV), C=N/C-N (284.90 eV), and C=O/C-O (287.45 eV) species.

3.2. Oxidase-Like Activity of Co-NC

The intrinsic oxidase-like activity of the Co-NC was first evaluated using three chromogenic substrates, namely TMB, OPD, and ABTS. Upon the addition of Co-NC, the originally colorless TMB, OPD, and ABTS solutions changed to blue, yellow, and green, with characteristic absorption peaks at 652, 450, and 415 nm, respectively (Figure 3a). These observations indicate that Co-NC was capable of catalyzing the oxidation of multiple substrates. The reaction conditions were then optimized. As depicted in Figure S4, the activity of Co-NC showed a typical bell-shaped dependence on both pH and temperature, with the maximum response observed at pH 4.0 and 40 °C. In addition, time-dependent absorbance measurements recorded at different catalyst concentrations showed that increasing the Co-NC dosage accelerated the reaction rate (Figure S5), indicating a concentration-dependent catalytic behavior.

To identify the active centers, pristine NC was used as a control. Compared with Co-NC, NC exhibited negligible catalytic activity toward TMB oxidation (Figure S6), indicating that the oxidase-like activity mainly originated from the atomically dispersed Co sites. This result was further supported by the KSCN poisoning experiment. As shown in Figure 3b, the oxidation of TMB was markedly suppressed after the addition of KSCN, confirming the essential role of Co-N_x sites. Unlike peroxidase mimics, oxidase-like catalysts relied on dissolved oxygen as the electron acceptor. As illustrated in Figure 3c, bubbling oxygen promoted the catalytic oxidation of TMB, whereas nitrogen purging significantly inhibited the reaction, indicating that dissolved O₂ participated directly in the catalytic process. Oxidase-like nanozymes generally activate O₂ to generate intermediate reactive oxygen species (ROS) during catalysis [40,41]. To clarify the reactive intermediates involved in this system, EPR spectroscopy was employed. As shown in Figure 3d, a characteristic 1:1:1:1 quartet assigned to DMPO-O₂^{•−} adduct was observed [40]. In contrast, no detectable signals corresponding to DMPO-•OH or TEMP-¹O₂ adducts were observed (Figure 3e,f). In addition, the peroxidase-based assay in Figure S7 demonstrated that no H₂O₂ was produced during catalysis. To further confirm the reactive species, scavenger experiments were performed using

CAT, IPA, Try, and SOD as scavengers for H_2O_2 , $\bullet\text{OH}$, $^1\text{O}_2$ and $\text{O}_2^{\bullet-}$ [42]. As shown in Figure 3g, only the addition of SOD led to an obvious decrease in absorbance, indicating that $\text{O}_2^{\bullet-}$ was the predominant reactive species. Based on these results, the oxidase-like activity of Co-NC can be attributed to the adsorption and activation of dissolved O_2 on isolated Co-N sites, which generates $\text{O}_2^{\bullet-}$ and thereby drives substrate oxidation (Figure 3h).

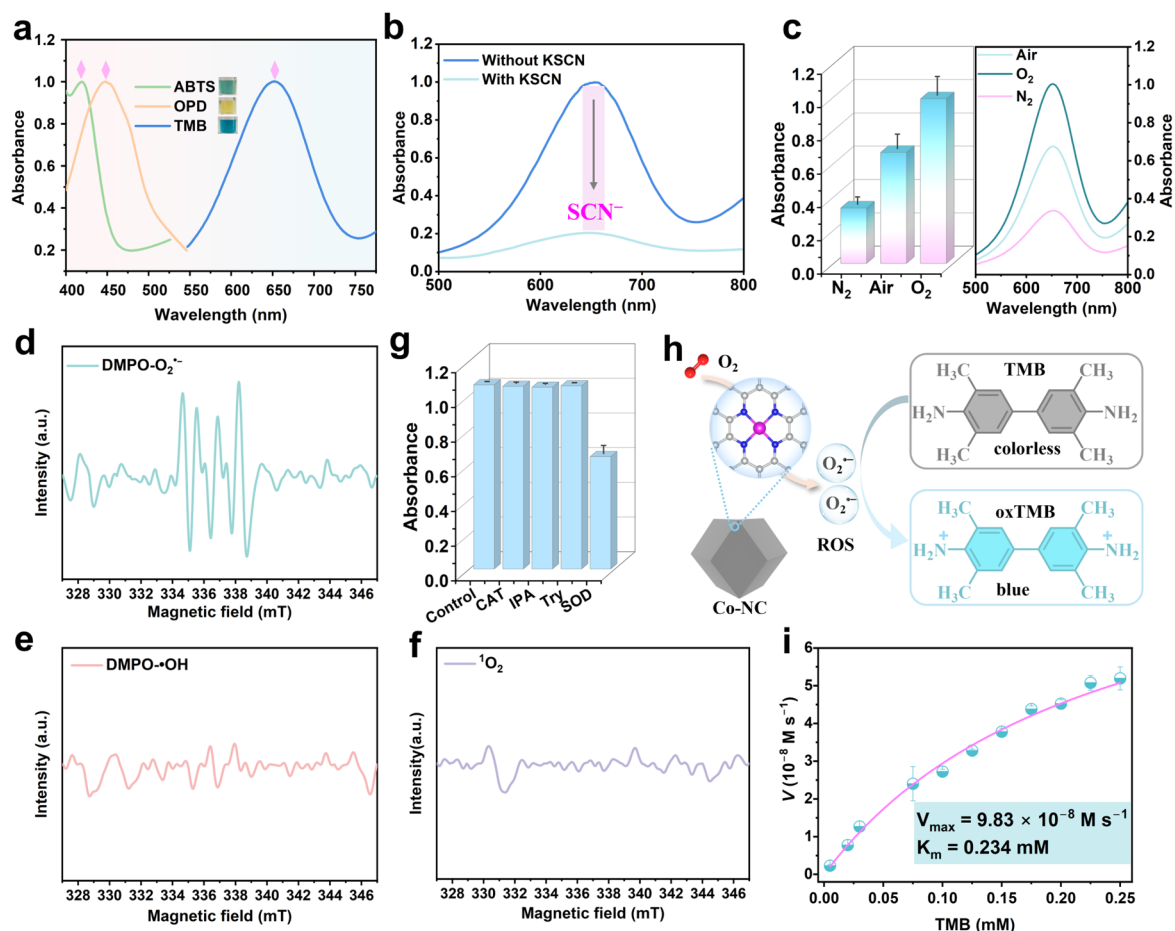


Figure 3. (a) The UV-vis absorption spectra of TMB, OPD and ABTS solutions after incubation with Co-NC. (b) Effect of KSCN on the Co-NC-catalyzed oxidation of TMB. (c) Absorbance and absorption spectra of Co-NC + TMB solutions under different gas-saturated conditions. EPR spectra of (d) DMPO- $\text{O}_2^{\bullet-}$, (e) DMPO- $\bullet\text{OH}$, and (f) TEMP- $^1\text{O}_2$ adducts. (g) Absorbance of Co-NC + TMB solutions in the presence of different scavengers. (h) Diagram of the catalytic mechanism. (i) Michaelis-Menten kinetics of the catalytic oxidation of TMB by Co-NC.

The steady-state kinetic behavior of Co-NC toward TMB oxidation was investigated as well. As shown in Figure 3i, the catalytic reaction followed typical Michaelis-Menten kinetics, and the corresponding Lineweaver-Burk plot exhibited good linearity (Figure S8). The K_m and V_{max} values were calculated to be 0.234 mM and $9.83 \times 10^{-8} \text{ M s}^{-1}$, respectively, indicating a favorable affinity for TMB and efficient catalytic activity (Table S1).

3.3. Reaction between HQ and 4-AAP for Light-On Detection of HQ

Based on the intrinsic oxidase-like activity of Co-NC, a “turn-on” colorimetric sensing platform was constructed through the oxidative coupling of HQ with 4-AAP. This design was inspired by the well-established oxidative coupling chemistry between phenolic compounds and 4-AAP [43], however, the reactivity of HQ toward 4-AAP under the present nanozyme-catalyzed conditions was experimentally investigated in this work. As depicted in Figure 4a, dissolved oxygen is activated on the Co-N_x sites to oxidize HQ into 1,4-benzoquinone, which subsequently couples with 4-AAP to form a pink quinone-imine product. To verify the feasibility of this design, a series of control experiments was performed. As presented in Figure 4b, the presence of HQ or 4-AAP alone in the Co-NC system produced no obvious absorption response. The mixture of HQ and 4-AAP without Co-NC also showed negligible absorbance. By contrast, the simultaneous addition of HQ and 4-AAP to the Co-NC system produced a pink solution with a distinct absorption peak at 507 nm. These observations confirmed the occurrence of the Co-NC-catalyzed oxidative coupling reaction.

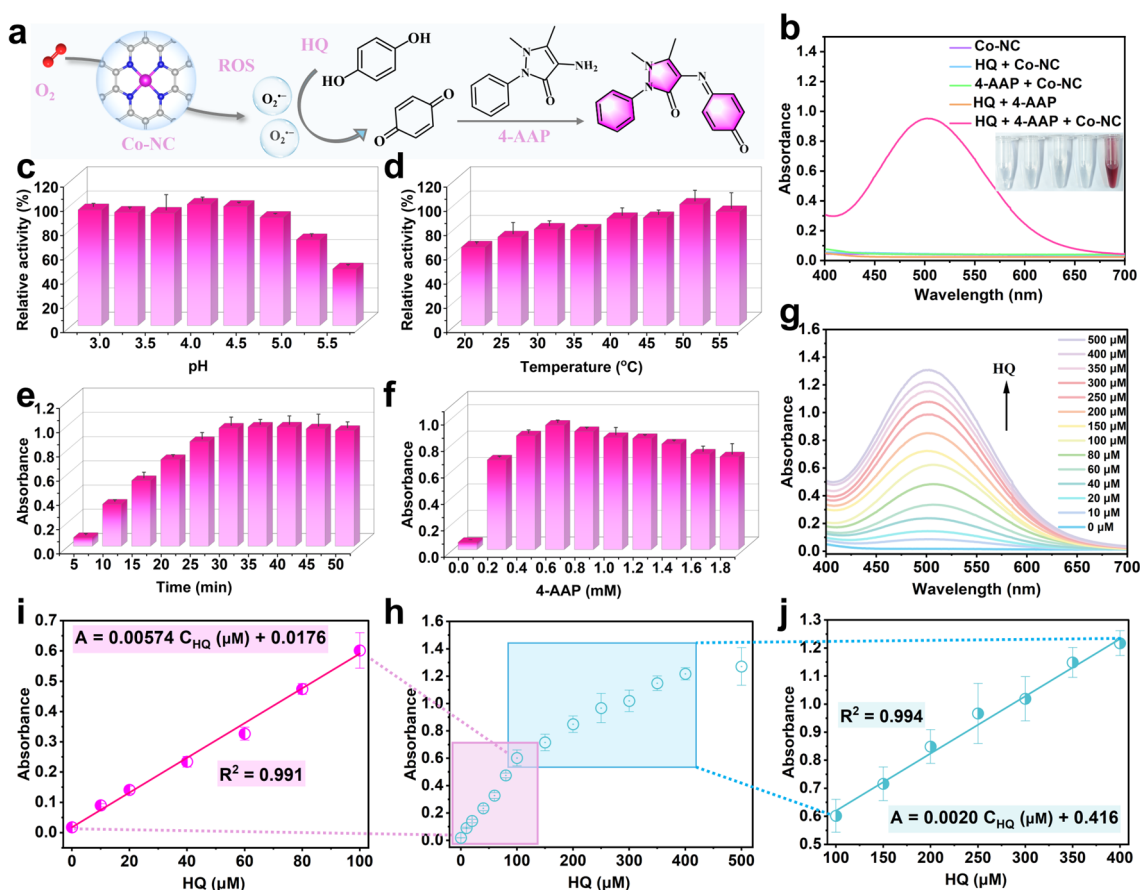


Figure 4. (a) Schematic illustration of the Co-NC-catalyzed reaction between HQ and 4-AAP. (b) UV-vis absorption spectra of the Co-NC system in the presence of HQ, 4-AAP, or HQ + 4-AAP. Effect of (c) pH, (d) temperature, (e) incubation time, and (f) 4-AAP concentration on the Co-NC-catalyzed reaction between HQ and 4-AAP. (g) UV-vis absorption spectra of the Co-NC + 4-AAP system in the presence of different concentrations of HQ. (h) Change in absorbance at 507 nm as a function of HQ concentration. (i,j) Linear ranges for HQ detection.

To achieve high sensitivity and reliable analytical performance, the sensing conditions were systematically optimized, as shown in Figure 4c–f. The system retained high catalytic activity over a pH range of 2.8–4.8, preserving more than 88% of the maximum response. The activity increased with temperature and then decreased, reaching a maximum at 50 °C while remaining above 80% between 30 and 55 °C. The reaction reached equilibrium within 30 min. In addition, the absorbance increased initially and then declined with increasing 4-AAP concentration, with an optimum value at 0.6 mM. Based on these results, pH 4.0, 0.6 mM 4-AAP, and a reaction time of 30 min were selected for the HQ sensing assay. Considering the need to match the enzymatic hydrolysis conditions in the cascade system, 37 °C was chosen as the operating temperature in the subsequent experiments.

Under the optimized conditions, the analytical performance of the sensing system for HQ detection was evaluated. As displayed in Figure 4g, the absorbance at 507 nm increased with increasing HQ concentration. The curve in Figure 4h showed good linear relationships between absorbance and HQ concentration over two concentration ranges (Figure 4i,j), namely 0–100 μ M ($R^2 = 0.991$) and 100–400 μ M ($R^2 = 0.994$). Based on the $3\sigma/S$ criterion, where σ is the standard deviation of blank signal and S is the slope of the calibration curve, the limit of detection (LOD) for HQ was calculated to be 0.26 μ M. Compared with recently reported HQ assays (Table S2), the present system adopts a turn-on colorimetric mode with low background interference, and its LOD is comparable to or lower than that of several reported methods. These results indicated that the proposed method provided sensitive and reliable detection of HQ.

Besides sensitivity, selectivity is also essential for practical application. Therefore, the selectivity of the Co-NC-based cascade platform was examined in the presence of potential interferents, including a structural analog (phenol) and common inorganic ions, such as, Na^+ , K^+ , Ca^{2+} , Zn^{2+} , Mg^{2+} , Cl^- , SO_4^{2-} , NO_3^- and CO_3^{2-} . In contrast to HQ, the addition of phenol or these inorganic ions caused only negligible changes in absorbance at 507 nm. The absence of obvious interference, particularly from the structural analog phenol, indicated that the oxidative coupling reaction

between HQ and 4-AAP proceeded with high specificity under the present assay conditions. These results demonstrated that the proposed method possessed reliable selectivity for HQ detection in complex matrices.

3.4. Detection of α -Glu Activity Based on Cascade Reactions

Based on the response of the system to HQ, an enzyme-nanozyme cascade colorimetric platform was established for α -Glu activity detection without the involvement of unstable H_2O_2 or additional enzymes. The sensing principle is illustrated in Figure 5a. In this cascade process, α -Glu specifically hydrolyzes α -Arbutin to produce HQ and glucose, and the generated HQ subsequently underwent Co-NC-catalyzed oxidative coupling with 4-AAP. As a result, the enzymatic hydrolysis event was converted into a measurable optical signal at 507 nm.

To examine the validity of this cascade strategy, the absorption spectra of different reaction mixtures were recorded (Figure 5b). Control systems containing Co-NC + 4-AAP with either α -Arbutin or α -Glu alone showed negligible absorbance at 507 nm. This result indicates that Co-NC did not directly generate a detectable signal in the absence of enzymatically produced HQ, thereby maintaining a low analytical background. By contrast, when α -Arbutin was pre-incubated with α -Glu before being introduced into the sensing system, a distinct absorption peak appeared at 507 nm, accompanied by a visible pink color. These observations confirm the successful execution of the tandem reaction, in which α -Glu-mediated hydrolysis was effectively coupled with the Co-NC-catalyzed oxidation process.

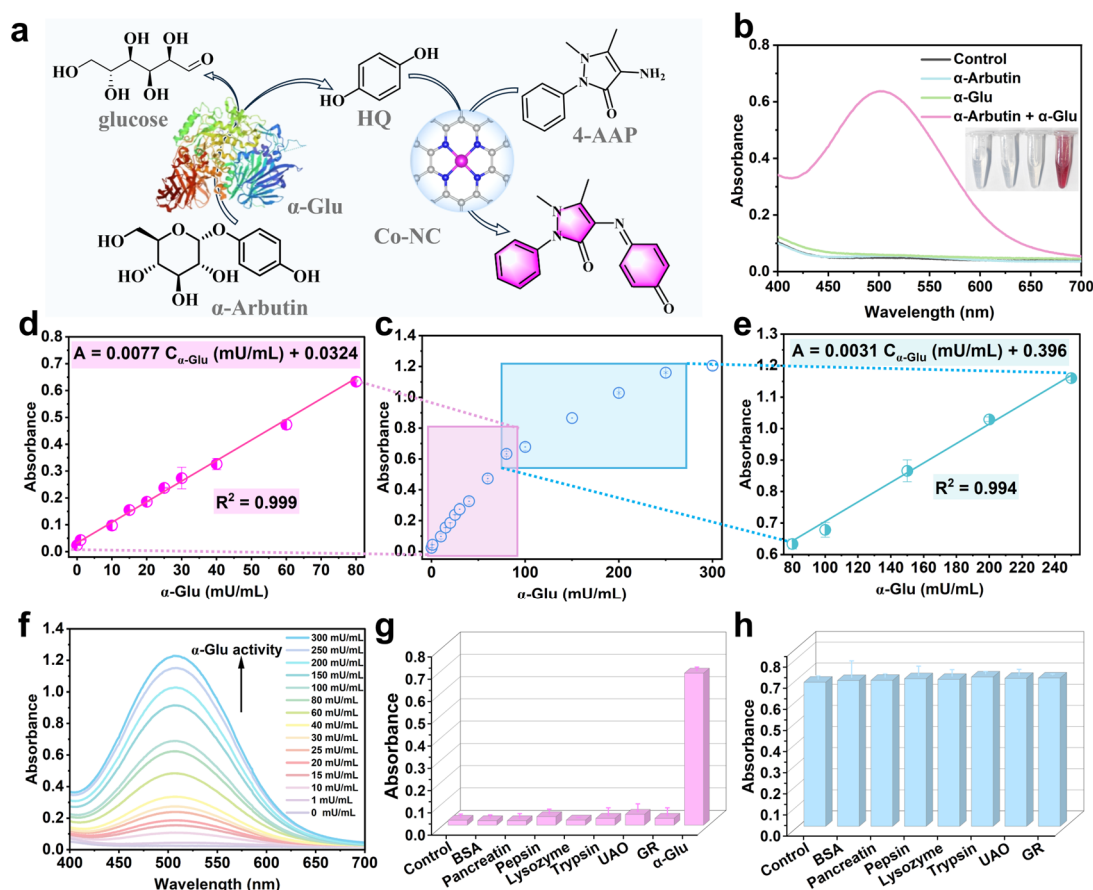


Figure 5. (a) Schematic illustration of the sensing mechanism for α -Glu activity assay based on the enzyme-nanozyme cascade reaction. (b) UV-vis absorption spectra of Co-NC + 4-AAP solutions in the presence of α -Arbutin, α -Glu, or α -Arbutin + α -Glu. (c) Change in absorbance at 507 nm as a function of α -Glu activity. (d,e) Linear ranges for α -Glu activity detection. (f) UV-vis absorption spectra of the Co-NC + 4-AAP + α -Arbutin system in the presence of different α -Glu activities. Absorbance changes induced by various proteins/enzymes in the (g) absence and (h) presence of α -Glu.

Before quantitative analysis, the conditions for the initial enzymatic hydrolysis step were optimized. The effects of hydrolysis time and the initial α -Arbutin concentration on the final absorbance were examined. As shown in Figure S10, the absorbance increased with incubation time and reached a plateau after 30 min, suggesting that the hydrolysis

reaction was essentially complete. In addition, an α -Arbutin concentration of 0.35 mM was selected as the optimal condition, as it provided sufficient substrate while avoiding significant background interference (Figure S11).

The analytical performance of the cascade platform was then evaluated under the optimized conditions. As shown in Figure 5c, the absorbance at 507 nm increased with increasing α -Glu activity. Consistent with this trend, the UV-vis absorption spectra in Figure 5f showed a gradual enhancement of the characteristic absorption signal as the α -Glu activity increased. The corresponding calibration plots (Figure 5d,e) exhibited two linear ranges of 1–80 mU/mL ($R^2 = 0.999$) and 80–250 mU/mL ($R^2 = 0.994$). The LOD was calculated to be 0.19 mU/mL. Compared to most of recently reported optical methods for α -Glu detection, this strategy demonstrates comparable or superior sensitivity (Table S3).

Selectivity is another important parameter for evaluating the reliability of the proposed biosensor. Therefore, the specificity of the proposed assay was evaluated by replacing α -Glu with several potentially interfering proteins and enzymes, including BSA, pancreatin, pepsin, lysozyme, trypsin, UAO, and GR. As shown in Figure 5g, the individual addition of these non-target species caused only negligible changes in the optical signal compared with blank group. Moreover, when these interferents were co-incubated with α -Glu, the system still exhibited a distinct response to the target analyte (Figure 5h). This anti-interference behavior is attributed to the specific recognition and hydrolysis of α -Arbutin by α -Glu, which enables accurate determination of α -Glu even in complex biological matrices.

To assess the practical applicability of the sensing platform, α -Glu activity was determined in serum samples using the standard addition method. As summarized in Table S4, the assay afforded recoveries ranging from 97.1% to 104.7%, with relative standard deviations (RSDs) of 1.3%–5.6%. These results indicate that the proposed method provided satisfactory accuracy and reproducibility for α -Glu determination in serum samples.

AGIs are oral antidiabetic drugs that reduce postprandial hyperglycemia by suppressing α -Glu catalyzed glucose production [44]. In addition, some AGIs have shown antiviral activity against pathogens such as HIV [45]. To assess the applicability of the proposed cascade platform for inhibitor screening, three widely used commercial drugs, acarbose, miglitol, and voglibose, were selected as model compounds. Upon binding to the active site of α -Glu, these inhibitors suppressed the hydrolysis of α -Arbutin, thereby reducing HQ generation and weakening the downstream Co-NC-catalyzed chromogenic reaction. Consequently, the absorption at 507 nm decreased in a concentration-dependent manner with increasing concentrations of acarbose (Figure 6a), miglitol (Figure 6b), and voglibose (Figure 6c). The corresponding dose-response curves are shown in Figure 6d–f, from which the half-maximal inhibitory concentrations (IC_{50}) of acarbose, miglitol, and voglibose were determined to be 5.2 μ M, 0.85 μ M and 0.31 μ M, respectively. These results provide a proof-of-concept demonstration that the proposed platform can be used to evaluate the inhibitory effects of representative commercial AGIs. Further validation using structurally diverse compound libraries and high-throughput assay formats will be required before its application as a comprehensive drug-screening platform.

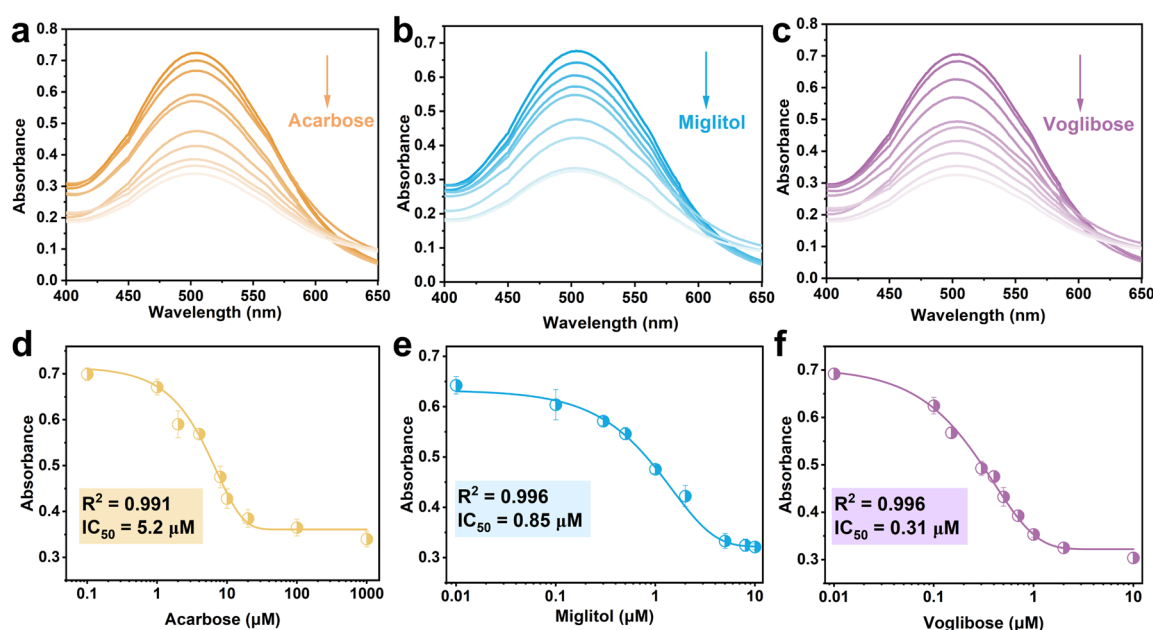


Figure 6. UV-vis absorption spectra of the α -Glu assay in the presence of different concentrations of (a) acarbose, (b) miglitol and (c) voglibose. The change of absorbance at 507 nm with the concentrations of (d) acarbose, (e) miglitol and (f) voglibose.

4. Conclusions

In summary, a Co-NC-based cascade colorimetric platform was developed for the determination of α -Glu activity and the screening of AGIs. By coupling the specific α -Glu-mediated hydrolysis of α -Arbutin with the Co-NC-catalyzed oxidative coupling of HQ and 4-AAP, the proposed system translates the enzymatic cleavage into a measurable pink optical signal. Unlike conventional three-enzyme cascade systems, this strategy does not rely on GOx or other auxiliary natural enzymes, which simplifies the assay design and avoids the instability associated with enzyme-coupled H₂O₂ generation. As a result, the platform enables the sensitive quantification of α -Glu with a detection limit of 0.19 mU/mL and maintains reliable analytical performance in serum samples, with recoveries of 97.1–104.7%. In addition, the system was successfully applied to the proof-of-concept evaluation of three representative commercial AGIs. These results indicate that the Co-NC-based cascade strategy is a practical tool for α -Glu-related biosensing and preliminary inhibitor evaluation.

Supplementary Materials

The additional data and information can be downloaded at: <https://media.sciltp.com/articles/others/2608251522400551/eChem-26060272-SI.docx>. Figure S1: TEM image of Co-NC. Figure S2: TEM images of (a-b) ZIF-8 and (c-d) Co@ ZIF-8. Figure S3: TEM images of NC. Figure S4: Effect of (a) pH and (b) temperature on the Co-NC catalyzed oxidation of TMB. Figure S5: Time-dependent absorbance changes of the reaction solutions at different Co-NC concentrations. Figure S6: UV-Vis absorption spectra of the TMB oxidation systems in the absence of catalyst (Control), and in the presence of pristine NC or Co-NC nanozyme. Figure S7: Verification of the non-H₂O₂ generation pathway during the Co-NC catalytic process. (a) Photographic illustration of the verification experiment. (b) The corresponding UV-Vis absorption spectra. The absence of the characteristic blue product (grey line) confirms that no free H₂O₂ was generated in the Co-NC-catalyzed oxidation system. The subsequent addition of external H₂O₂ triggered a distinct colorimetric response (blue line), serving as a positive control. Figure S8: Lineweaver-Burk double reciprocal plot for the Co-NC-catalyzed oxidation of TMB. Table S1: Kinetic parameters of the Co-NC and other Co-based catalysts. Table S2: Comparison of various HQ assays. Figure S9: The absorbance at 507 nm recorded in the presence of HQ, phenol, and various interfering inorganic ions. Figure S10: Effect of the enzymatic hydrolysis time on the absorbance of the cascade sensing system. Figure S11: Effect of the enzymatic α -Arbutin concentration on the absorbance of the cascade sensing system. Table S3: Comparison of various methods for the determination of α -Glu. Table S4: Determination of α -Glu activity in real samples. References [46–73] are cited in supplementary materials.

Author Contributions

C.C.: Conceptualization, Methodology, Validation, Resources, Formal analysis, Investigation, Supervision, Project administration, Funding acquisition, Data Curation, Visualization, Writing—Original Draft, Writing—Review & Editing. T.X.: Investigation, Resources, Data Curation. X.G.: Resources, Data Curation. Z.H.: Resources, Data Curation. Y.L. (Yawen Liu): Resources, Data Curation. F.Z.: Resources, Data Curation. G.W.: Writing—Review & Editing. Y.L. (Yizhong Lu): Writing—Review & Editing, Funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

Data will be made available on request.

Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

References

1. Huang, S.; Yang, E.; Yao, J.; et al. Carbon Dots Doped with Nitrogen and Boron as Ultrasensitive Fluorescent Probes for Determination of α -Glucosidase Activity and Its Inhibitors in Water Samples and Living Cells. *Microchim. Acta* **2018**, *185*, 394. <https://doi.org/10.1007/s00604-018-2932-1>.
2. Li, J.; He, G.; Wang, B.; et al. Fabrication of Reusable Electrochemical Biosensor and Its Application for the Assay of α -Glucosidase Activity. *Anal. Chim. Acta* **2018**, *1026*, 140–146. <https://doi.org/10.1016/j.aca.2018.04.015>.
3. Nsanamahoro, S.; Wang, W.F.; Zhang, Y.; et al. α -Glucosidase-Triggered Reaction for Fluorometric and Colorimetric Assays Based on the Formation of Silicon-Containing Nanoparticles. *Anal. Chem.* **2021**, *93*, 15412–15419. <https://doi.org/10.1021/acs.analchem.1c03210>.
4. Zhong, Y.Y.; Chen, H.S.; Wu, P.P.; et al. Synthesis and Biological Evaluation of Novel Oleanolic Acid Analogues as Potential α -Glucosidase Inhibitors. *Eur. J. Med. Chem.* **2019**, *164*, 706–716. <https://doi.org/10.1016/j.ejmech.2018.12.046>.
5. Zhong, Y.; Li, Q.L.; Lu, M.; et al. A Colorimetric Sensing Strategy Based on Enzyme@Metal-Organic Framework and Oxidase-like $\text{IrO}_2/\text{MnO}_2$ Nanocomposite for α -Glucosidase Inhibitor Screening. *Microchim. Acta* **2020**, *187*, 675. <https://doi.org/10.1007/s00604-020-04660-6>.
6. Zhang, Y.; Li, M.; Li, S.; et al. Two-Dimensional Metal-Organic Framework Catalyzed Chemiluminescent Reaction for Alpha-Glucosidase Inhibitor Screening. *Talanta* **2023**, *264*, 124748. <https://doi.org/10.1016/j.talanta.2023.124748>.
7. Wan, G.Z.; Chen, J. Cerium-Based Metal–Organic Framework Nanocrystals for Colorimetric Screening of α -Glucosidase Inhibitors. *ACS Appl. Nano Mater.* **2024**, *7*, 13636–13648. <https://doi.org/10.1021/acsanm.4c02151>.
8. Wei, H.; Xu, F.; Xu, T.; et al. A Nanoprobe-Integrated Capillary Sensing System for Highly Sensitive SERS Detection of Neutral α -Glucosidase in Semen. *Int. J. Biol. Macromol.* **2025**, *317*, 144847. <https://doi.org/10.1016/j.ijbiomac.2025.144847>.
9. Guo, P.C.; Shen, H.D.; Fang, J.J.; et al. On-Line High-Performance Liquid Chromatography Coupled with Biochemical Detection Method for Screening of α -Glucosidase Inhibitors in Green Tea. *Biomed. Chromatogr.* **2018**, *32*, e4281. <https://doi.org/10.1002/bmc.4281>.
10. Wu, D.; Zhao, Q.; Zhang, B.; et al. Iron-Doped Polymer Dots with Enhanced Fluorescence and Dual Enzyme Activity for Versatile Bioassays. *Anal. Chem.* **2024**, *96*, 2929–2938. <https://doi.org/10.1021/acs.analchem.3c04514>.
11. Kang, G.; Liu, W.; Liu, F.; et al. Single-Atom Pt Catalysts as Oxidase Mimic for p-Benzoquinone and α -Glucosidase Activity Detection. *Chem. Eng. J.* **2022**, *449*, 137855. <https://doi.org/10.1016/j.cej.2022.137855>.
12. Zhang, C.; Chen, C.; Zhao, D.; et al. Multienzyme Cascades Based on Highly Efficient Metal–Nitrogen–Carbon Nanozymes for Construction of Versatile Bioassays. *Anal. Chem.* **2022**, *94*, 3485–3493. <https://doi.org/10.1021/acs.analchem.1c04018>.
13. Li, S.; Zhang, H.; Huang, Z.; et al. Fluorometric and Colorimetric Dual-Mode Sensing of α -Glucosidase Based on Aggregation-Induced Emission Enhancement of AuNCs. *J. Mater. Chem. B* **2024**, *12*, 1550–1557. <https://doi.org/10.1039/d3tb02532b>.
14. Lee, T.; Park, J.; Oh, S.H.; et al. Glucose Oxidase Activity Colorimetric Assay Using Redox-Sensitive Electrochromic Nanoparticle-Functionalized Paper Sensors. *ACS Omega* **2024**, *9*, 15493–15501. <https://doi.org/10.1021/acsomega.4c00335>.
15. Bezuneh, T.T.; Nan, F.; Li, X.; et al. Self-Cascading Nucleotide-Based Nanozyme for Ultra-Sensitive Dual-Signal Detection of α -Glucosidase Activity. *Talanta* **2026**, *297*, 128726. <https://doi.org/10.1016/j.talanta.2025.128726>.
16. Wang, Y.; Zhao, Q.; Liu, T.; et al. Recent Advances in Nanozyme-Based Biosensors for the Detection of Disease-Related Enzymes. *TrAC Trends Anal. Chem.* **2026**, *201*, 118912. <https://doi.org/10.1016/j.trac.2026.118912>.
17. Yan, M.; Meng, T.; Bao, X.; et al. In Situ Domain-Confined CoIrOx Clusters within MOFs: Efficient Artificial Nanozymes for Multimodal Sensing. *ACS Nano* **2026**, *20*, 12524–12536. <https://doi.org/10.1021/acsnano.6c00731>.
18. Baldim, V.; Yadav, N.; Bia, N.; et al. Polymer-Coated Cerium Oxide Nanoparticles as Oxidoreductase-like Catalysts. *ACS Appl. Mater. Interfaces* **2020**, *12*, 42056–42066. <https://doi.org/10.1021/acsami.0c08778>.
19. Dai, S.; Jiang, L.; Liu, L.; et al. MOF-Encapsulated Copper-Doped Carbon Dots Nanozymes with Excellent Biological Activity Promote Diabetes Wound Healing. *Regen. Biomater.* **2024**, *11*, rbae119. <https://doi.org/10.1093/rb/rbae119>.
20. Zhao, Y.; Cheng, J.; Li, Z.; et al. Nanozymes in Biomedical Applications: Innovations Originated from Metal-Organic Frameworks. *Adv. Healthc. Mater.* **2025**, *14*, e2402066. <https://doi.org/10.1002/adhm.202402066>.
21. Yan, L.; Cao, Z.; Ren, L.; et al. A Sonoresponsive and NIR-II-Photoresponsive Nanozyme for Heterojunction-Enhanced “Three-in-One” Multimodal Oncotherapy. *Adv. Healthc. Mater.* **2024**, *13*, e2302190. <https://doi.org/10.1002/adhm.202302190>.
22. Zeng, D.; Luo, W.; Li, J.; et al. Gold Nanoparticles-Based Nanoconjugates for Enhanced Enzyme Cascade and Glucose Sensing. *Analyst* **2012**, *137*, 4435–4439. <https://doi.org/10.1039/c2an35900f>.
23. Jiang, Y.; Chen, Z.; Yuan, Y.; et al. Cu–N–C Single-Atom Nanozyme as an Ultrasensitive Sensing Platform for α -Glucosidase Detection. *Mater. Today Chem.* **2024**, *41*, 102327. <https://doi.org/10.1016/j.mtchem.2024.102327>.
24. Qi, M.; Ye, Y.; Xu, Y.; et al. Metal Nanoclusters Engineered Interfacial Adsorption for Enhanced ROS Independent Oxidase-Mimicking Activity. *Nano Res.* **2026**, *19*, 94908241. <https://doi.org/10.26599/nr.2025.94908241>.

25. He, X.Y.; Shen, Q.W.; Zhu, C.J.; et al. Crosstalk-Free Dual-Mode Biosensing in a Single Mof for α -Glucosidase Activity Monitoring and Inhibitor Screening. *Talanta* **2026**, *297*, 128693. <https://doi.org/10.1016/j.talanta.2025.128693>.
26. Fu, Q.; Liang, S.; Zhang, S.; et al. Boron-Doped G-C₃N₄ Supporting Cu Nanozyme for Colorimetric-Fluorescent-Smartphone Detection of α -Glucosidase. *Anal. Chim. Acta* **2024**, *1311*, 342715. <https://doi.org/10.1016/j.aca.2024.342715>.
27. Cui, L.; Yang, Y.; Jiang, S.; et al. Exogenous Co-Reactant-Free Electrochemiluminescent Biosensor for Ratiometric Measurement of α -Glucosidase Based on a ZIF-67-Regulated Hydrogen-Bonded Organic Framework. *ACS Sensors* **2024**, *9*, 1023–1030. <https://doi.org/10.1021/acssensors.4c00036>.
28. Wang, Y.; Zhao, Q.; Fang, Q.; et al. A Cascade Nanoreactor Based on Metal Azolate Framework Integrated Natural Enzyme for α -Glucosidase Activity Assay and Inhibitor Screening. *J. Colloid Interface Sci.* **2025**, *695*, 137764. <https://doi.org/10.1016/j.jcis.2025.137764>.
29. Liu, G.; Wang, L.; Zhu, F.; et al. Facile Construction of a Reusable Multi-Enzyme Cascade Bioreactor for Effective Fluorescence Discrimination and Quantitation of Amino Acid Enantiomers. *Chem. Eng. J.* **2022**, *428*, 131975. <https://doi.org/10.1016/j.cej.2021.131975>.
30. Liu, S.; Nie, C.; He, F.; et al. Oxidase-like Nanozymes-Driven Colorimetric, Fluorescence and Electrochemiluminescence Assays for Pesticide Residues. *Trends Food Sci. Technol.* **2024**, *150*, 104597. <https://doi.org/10.1016/j.tifs.2024.104597>.
31. Li, J.; Cai, X.; Jiang, P.; et al. Co-Based Nanozymatic Profiling: Advances Spanning Chemistry, Biomedical, and Environmental Sciences. *Adv. Mater.* **2024**, *36*, 2307337. <https://doi.org/10.1002/adma.202307337>.
32. Li, Z.; Liu, F.; Chen, C.; et al. Regulating the N Coordination Environment of Co Single-Atom Nanozymes for Highly Efficient Oxidase Mimics. *Nano Lett.* **2023**, *23*, 1505–1513. <https://doi.org/10.1021/acs.nanolett.2c04944>.
33. Liu, F.; Li, Z.; Wei, H.; et al. Coordinatively Unsaturated Cobalt Single-Atom Nanozymes for Visual Pesticides Detection by Smartphone-Based Platform. *Nano Res.* **2024**, *17*, 2298–2307. <https://doi.org/10.1007/s12274-023-6039-6>.
34. Xu, P.; Liu, W.; Jiang, Y.; et al. Unraveling the Proximity Effects of a Co–P Dual Site in Phosphorus-Coordinated Cobalt Single-Atom Nanozymes. *ACS Nano* **2025**, *19*, 41911–41922. <https://doi.org/10.1021/acsnano.5c18031>.
35. Jiang, B.; Duan, D.; Gao, L.; et al. Standardized Assays for Determining the Catalytic Activity and Kinetics of Peroxidase-like Nanozymes. *Nat. Protoc.* **2018**, *13*, 1506–1520. <https://doi.org/10.1038/s41596-018-0001-1>.
36. He, Y.; Hwang, S.; Cullen, D.A.; et al. Highly Active Atomically Dispersed CoN₄ Fuel Cell Cathode Catalysts Derived from Surfactant-Assisted MOFs: Carbon-Shell Confinement Strategy. *Energy Environ. Sci.* **2019**, *12*, 250–260. <https://doi.org/10.1039/c8ee02694g>.
37. Liu, P.; Li, Y.; Sun, C.; et al. Atomic-Level Co/Mesoporous Carbon Nanofibers for Efficient Electrochemical H₂O₂ Production. *ACS Appl. Nano Mater.* **2025**, *8*, 7267–7277. <https://doi.org/10.1021/acsanm.5c00621>.
38. Yang, M.; Cheng, X.H.; Geng, F.T.; et al. Anchoring Co-Fe Alloy Nano-Grains on Carbon Fibers by an In Situ Alloying Strategy to Boost the Catalytic Performance for Rapid Oxidative Degradation of Emerging Contaminants. *J. Mater. Chem. A* **2025**, *13*, 4329–4342. <https://doi.org/10.1039/d4ta08657k>.
39. Shu, Y.; Li, Z.; Yang, Y.; et al. Isolated Cobalt Atoms on N-Doped Carbon as Nanozymes for Hydrogen Peroxide and Dopamine Detection. *ACS Appl. Nano Mater.* **2021**, *4*, 7954–7962. <https://doi.org/10.1021/acsanm.1c01278>.
40. Zhang, F.; Liu, W.; Hao, Z.; et al. Defect-Engineered Iron Single-Site Catalysts with Tailored Atomic Coordination for Enhanced Mild Photothermal Therapy via Triad Modulation of Apoptosis and Ferroptosis. *BMEMat* **2026**, *4*, e70051. <https://doi.org/10.1002/bmm2.70051>.
41. Chen, C.; Liu, W.; Ni, P.; et al. Engineering Two-Dimensional Pd Nanoplates with Exposed Highly Active {100} Facets toward Colorimetric Acid Phosphatase Detection. *ACS Appl. Mater. Interfaces* **2019**, *11*, 47564–47570. <https://doi.org/10.1021/acsam.9b16279>.
42. Zhu, S.; Li, Z.; Zhang, F.; et al. Single-Atom Cobalt Catalysts as Highly Efficient Oxidase Mimics for Time-Based Visualization Monitoring the TAC of Skin Care Products. *Chem. Eng. J.* **2023**, *456*, 141053. <https://doi.org/10.1016/j.cej.2022.141053>.
43. Wu, S.; Guo, D.; Xu, X.; et al. Colorimetric Quantification and Discrimination of Phenolic Pollutants Based on Peroxidase-like Fe₃O₄ Nanoparticles. *Sens. Actuat. B Chem.* **2020**, *303*, 127225. <https://doi.org/10.1016/j.snb.2019.127225>.
44. Atta-ur, R.; Zareen, S.; Choudhary, M.I.; et al. α -Glucosidase Inhibitory Activity of Triterpenoids from Cichorium Intybus. *J. Nat. Prod.* **2008**, *71*, 910–913. <https://doi.org/10.1021/np800001v>.
45. Simoes-Pires, C.A.; Hmicha, B.; Marston, A.; et al. A TLC Bioautographic Method for the Detection of Alpha- and Beta-Glucosidase Inhibitors in Plant Extracts. *Phytochem. Anal.* **2009**, *20*, 511–515. <https://doi.org/10.1002/pca.1154>.
46. Keoingthong, P.; Li, S.; Zhu, Z.; et al. Enzyme-Mimic Activity Study of Superstable and Ultrasmall Graphene Encapsulated CoRu Nanocrystal. *APL Mater.* **2021**, *9*, 051110. <https://doi.org/10.1063/5.0048777>.
47. Qin, W.; Su, L.; Yang, C.; et al. Colorimetric Detection of Sulfite in Foods by a TMB–O₂–Co₃O₄ Nanoparticles Detection System. *J. Agric. Food Chem.* **2014**, *62*, 5827–5834. <https://doi.org/10.1021/jf500950p>.
48. Huang, L.; Chen, K.; Zhang, W.; et al. ssDNA-Tailorable Oxidase-Mimicking Activity of Spinel MnCo₂O₄ for Sensitive Biomolecular Detection in Food Sample. *Sens. Actuators B Chem* **2018**, *269*, 79–87. <https://doi.org/10.1016/j.snb.2018.04.150>.

49. Gao, L.; Zhuang, J.; Nie, L.; et al. Intrinsic Peroxidase-like Activity of Ferromagnetic Nanoparticles. *Nat. Nanotechnol.* **2007**, *2*, 577–583. <https://doi.org/10.1038/nnano.2007.260>.
50. Zhu, H.; Quan, Z.; Hou, H.; et al. A Colorimetric Immunoassay Based on Cobalt Hydroxide Nanocages as Oxidase Mimics for Detection of Ochratoxin A. *Anal. Chim. Acta* **2020**, *1132*, 101–109. <https://doi.org/10.1016/j.aca.2020.07.068>.
51. Chen, Y.; Cao, H.; Shi, W.; et al. Fe–Co Bimetallic Alloy Nanoparticles as a Highly Active Peroxidase Mimetic and Its Application in Biosensing. *Chem. Commun.* **2013**, *49*, 5013–5015. <https://doi.org/10.1039/c3cc41569d>.
52. Su, L.; Dong, W.; Wu, C.; et al. The Peroxidase and Oxidase-like Activity of NiCo₂O₄ Mesoporous Spheres: Mechanistic Understanding and Colorimetric Biosensing. *Anal. Chim. Acta* **2017**, *951*, 124–132. <https://doi.org/10.1016/j.aca.2016.11.035>.
53. Dong, W.; Zhuang, Y.; Li, S.; et al. High Peroxidase-like Activity of Metallic Cobalt Nanoparticles Encapsulated in Metal–Organic Frameworks Derived Carbon for Biosensing. *Sens. Actuators B Chem.* **2018**, *255*, 2050–2057. <https://doi.org/10.1016/j.snb.2017.09.013>.
54. Chen, J.; Zheng, X.; Zhang, J.; et al. Bubble-Templated Synthesis of Nanocatalyst Co/C as NADH Oxidase Mimic. *Natl. Sci. Rev.* **2022**, *9*, nwab186. <https://doi.org/10.1093/nsr/nwab186>.
55. Li, S.; Hou, Y.; Chen, Q.; et al. Promoting Active Sites in MOF-Derived Homobimetallic Hollow Nanocages as a High-Performance Multifunctional Nanozyme Catalyst for Biosensing and Organic Pollutant Degradation. *ACS Appl. Mater. Interfaces* **2020**, *12*, 2581–2590. <https://doi.org/10.1021/acsami.9b20275>.
56. Chen, Y.; Liu, X.; Zhang, S.; et al. Ultrasensitive and Simultaneous Detection of Hydroquinone, Catechol and Resorcinol Based on the Electrochemical Co-Reduction Prepared Au-Pd Nanoflower/Reduced Graphene Oxide Nanocomposite. *Electrochim. Acta* **2017**, *231*, 677–685. <https://doi.org/10.1016/j.electacta.2017.02.060>.
57. Yuan, J.; Guo, W.; Wang, E. Utilizing a CdTe Quantum Dots–Enzyme Hybrid System for the Determination of Both Phenolic Compounds and Hydrogen Peroxide. *Anal. Chem.* **2008**, *80*, 1141–1145. <https://doi.org/10.1021/ac0713048>.
58. Wu, T.; Ma, Z.; Li, P.; et al. Bifunctional Colorimetric Biosensors via Regulation of the Dual Nanoenzyme Activity of Carbonized FeCo-ZIF. *Sens. Actuators B Chem.* **2019**, *290*, 357–363. <https://doi.org/10.1016/j.snb.2019.03.130>.
59. Xing, N.; Yang, L.; Wang, L.; et al. Cu/Mn Synergy Catalysis-Based Colorimetric Sensor for Visual Detection of Hydroquinone. *Coatings* **2024**, *14*, 453. <https://doi.org/10.3390/coatings14040453>.
60. Wang, Z.; Ren, Q.; Han, L.; et al. Synergistic Electronic Modulation in Cysteine-Functionalized CoFeMn-LDH Heterophase Nanozymes: Multi-Interface Engineering for Smartphone-Enabled Ultrasensitive Hydroquinone Sensing. *Chem. Eng. J.* **2025**, *520*, 165749. <https://doi.org/10.1016/j.cej.2025.165749>.
61. Song, H.; Liu, F.; Wang, T.; et al. Peroxidase-like Activity of Iron-Doped Carbon Dots with Efficient ¹O₂ Production for Colorimetric and Photothermal Dual-Mode Detection of α -Glucosidase and Its Inhibitors. *Microchem. J.* **2025**, *216*, 114830. <https://doi.org/10.1016/j.microc.2025.114830>.
62. Cheng, X.; Xu, J.; Wang, L.; et al. A Redox Modulated Ratiometric Fluorometric Method Based on the Use of Dual-Color Carbon Dots for Determination of the Activity of Enzymes Participating in Ascorbic Acid-Related Reactions. *Microchim. Acta* **2019**, *186*, 818. <https://doi.org/10.1007/s00604-019-3820-z>.
63. Liu, J.; Duan, X.; Wang, M.; et al. A Label-Free Fluorescent Sensor Based on Silicon Quantum Dots–MnO₂ Nanosheets for the Detection of α -Glucosidase and Its Inhibitor. *Anal.* **2019**, *144*, 7398–7405. <https://doi.org/10.1039/c9an01680e>.
64. Yue, X.; Su, L.; Chen, X.; et al. A New Method for the in Situ Assay of α -Glucosidase Activity and Inhibitor Screening through an Enzyme Substrate-Mediated DNA Hybridization Chain Reaction. *New J. Chem.* **2019**, *43*, 9458–9465. <https://doi.org/10.1039/c9nj01868a>.
65. Zhang, J.; Liu, J.; Wang, M.; et al. A Fluorometric Assay for α -Glucosidase Activity Based on Quaternary AgInZnS QDs. *Microchim. Acta* **2021**, *188*, 227. <https://doi.org/10.1007/s00604-021-04855-5>.
66. Shi, M.; Cen, Y.; Xu, G.; et al. Ratiometric Fluorescence Monitoring of α -Glucosidase Activity Based on Oxidase-like Property of MnO₂ Nanosheet and Its Application for Inhibitor Screening. *Anal. Chim. Acta* **2019**, *1077*, 225–231. <https://doi.org/10.1016/j.aca.2019.05.037>.
67. Tang, C.; Qian, Z.; Qian, Y.; et al. A Fluorometric and Real-Time Assay for α -Glucosidase Activity through Supramolecular Self-Assembly and Its Application for Inhibitor Screening. *Sens. Actuators B Chem.* **2017**, *245*, 282–289. <https://doi.org/10.1016/j.snb.2017.01.150>.
68. Kong, W.; Wu, D.; Xia, L.; et al. Carbon Dots for Fluorescent Detection of α -Glucosidase Activity Using Enzyme Activated Inner Filter Effect and Its Application to Anti-Diabetic Drug Discovery. *Anal. Chim. Acta* **2017**, *973*, 91–99. <https://doi.org/10.1016/j.aca.2017.03.050>.
69. Ao, H.; Feng, H.; Huang, X.; et al. A Reversible Fluorescence Nanoswitch Based on Dynamic Covalent B–O Bonds Using Functional Carbon Quantum Dots and Its Application for α -Glucosidase Activity Monitoring. *J. Mater. Chem. C* **2017**, *5*, 2826–2832. <https://doi.org/10.1039/c7tc00223h>.
70. Zhang, H.; Wang, Z.; Yang, X.; et al. The Determination of α -Glucosidase Activity through a Nano Fluorescent Sensor of F-PDA–CoOOH. *Anal. Chim. Acta* **2019**, *1080*, 170–177. <https://doi.org/10.1016/j.aca.2019.07.014>.

71. Lv, Y.; Zhou, C.; Li, M.; et al. A Dual-Mode Sensing System Based on Carbon Quantum Dots and Fe Nanozymes for the Detection of α -Glucosidase and Its Inhibitors. *Talanta* **2024**, *268*, 125328. <https://doi.org/10.1016/j.talanta.2023.125328>.
72. Chen, H.; Zhang, J.; Wu, H.; et al. Sensitive Colorimetric Assays for α -Glucosidase Activity and Inhibitor Screening Based on Unmodified Gold Nanoparticles. *Anal. Chim. Acta* **2015**, *875*, 92–98. <https://doi.org/10.1016/j.aca.2015.02.022>.
73. Zhang, J.; Liu, Y.; Lv, J.; et al. A Colorimetric Method for α -Glucosidase Activity Assay and Its Inhibitor Screening Based on Aggregation of Gold Nanoparticles Induced by Specific Recognition between Phenylenediboric Acid and 4-Aminophenyl- α -D-Glucopyranoside. *Nano Res.* **2014**, *8*, 920–930. <https://doi.org/10.1007/s12274-014-0573-1>.