Supplementary Information (SI) for ChemComm.
This journal is © The Royal Society of Chemistry 2026

Supplementary information

The prenucleation clusters of ZnSe magic size clusters mimicking natural hydrolase

Yang Li,*[a†] Yujie Yang,[a†] Yue Yang,[a†] Xieruoying Zhong,[a] Xianxiang Wang[a] and Li He*[a]

[a] College of Science, Sichuan Agricultural University, Xin Kang Road, Yucheng District, Yaan, 625014, China

[†] These authors contribute equally

Email: li_yang@sicau.edu.cn (Y. Li), lihe@sicau.edu.cn (L. He)

Experimental section

Materials

Znic chloride ($ZnCl_2$, 98%) was purchased from Shanghai Acmec Biochemical Co., Lid. (Shanghai, China). 3-mercaptopropionic acid (MPA, 99%), 1-butylamine (BTA, AR), L-cystenine (L-cys, 99%), 4-nitrophenyl acetate (p-NPA, 98%) were purchased from Shanghai Titan Scientific Co., Ltd. (Shanghai, China). Selenourea (98%) was obtained from Sigma-Aldrich (USA). Hydrogen peroxide (H_2O_2, 30%), copper(Ⅱ) chloride dihydrate ($CuCl_2 \cdot 2H_2O$, AR), sodium hydroxide, acetic acid (HAc, AR), sodium acetate anhydrous (NaAc, AR), and sodium hydroxide (NaOH, AR), 3,3',5,5'-tetramethylbenzidine (TMB, 98%), tris(Hydroxymethyl)Aminomethane Hydrochloride (Tris-HCl, AR) were supplied by Chengdu Cologne Chemical Co., Ltd. (Chengdu, China). All the materials were applied without further purification.

Characterization

X-ray photoelectron spectroscopy (XPS) analysis was obtained by an Escalab 250 Xi XPS system with Al Kα source (hv = 1486.6 eV). X-ray diffraction (XRD) patterns were obtained using an X-ray diffractometer (DX-2700, Dandong, China) using Cu-Kα radiation. Fourier transform infrared spectrometer (FT-IR) characterization was performed on a Nicolet iS20 (USA). UV–vis spectrophotometer (AOE, A390, China) and enzyme-labeled instrument (MD, SpectraMax190, USA) were used to collect the data of the catalytic performance.

Synthesis of ZnSe prenucleation clusters

0.4 mmol of $ZnCl_2$ (54.5 mg) and 0.8 mmol of MPA (70 μL) were mixed in 18 mL of water, and the pH was adjusted to 12 using 5 M NaOH. The volume was then brought to 19.5 mL by adding water. Under stirring, 0.5 mL of 200 mM SeU (0.1 mmol) was added, and the reaction proceeded at room temperature (~25 °C) for 5 hours. The product was purified by centrifugation and washing with ethanol. The final product was obtained by freeze-drying.

CA mimetic activity assessment

The carbonic anhydrase-like activity of ZnSe was measured using p-NPA as the substrate by UV spectrophotometry. Specifically, 50 μL of ZnSe solution (8 mg/mL) and 20 μL of p-NPA solution (10 mM) were added to 930 μL of Tris-HCl buffer (pH 10.0), resulting in a total reaction volume of 1 mL. After incubation at 25 °C for 30 minutes, the mixture was diluted 6-fold and scanned for absorbance between 300–500 nm using a UV spectrophotometer. A blank control was prepared by replacing the ZnSe solution with an equal volume of ultrapure water, containing only p-NPA.

Determination of steady-state kinetic parameters

To determine the kinetic parameters of ZnSe-catalyzed hydrolysis of p-NPA, the initial reaction rate (V_0) was measured at various substrate concentrations. Briefly, 20 μL of p-NPA at different concentrations (0.02–5 mM) was mixed with 50 μL of ZnSe solution (20 mg/mL) and 930 μL of Tris-HCl buffer (pH 10.0). After 30 minutes of reaction at 25 °C, the mixture was diluted 6-fold, and the absorbance at 400 nm was measured to calculate V_o according to Equation (1):

$$V_o = [\Delta A_{400\,nm} / (\varepsilon L)] / t \qquad (1)$$

where ε is the molar extinction coefficient of the product p-nitrophenol (p-NP) (18,300 $M^{-1}\cdot cm^{-1}$), L is the path length (1 cm), and t is the reaction time (30 minutes).

The V_o values obtained at different substrate concentrations [S] were fitted using the Michaelis–Menten equation (Equation 2) via nonlinear regression and the Lineweaver–Burk equation (Equation 3) via linear regression:

$$V_o = (V_{max} \times [S]) / (K_m + [S]) \qquad (2)$$

$$1/V_o = (K_m / V_{max}) \times (1 / [S]) + 1 / V_{max} \quad (3)$$

From these fittings, the apparent maximum reaction rate (V_{max}) and apparent Michaelis constant (K_m) were determined.

Quantification of the concentration of Cu^{2+}

The introduction of Cu^{2+} induces a concomitant decrease in the carbonic anhydrase (CA)-like activity of ZnSe cluster and an increase in the peroxidase-like activity of the resulting $Cu_{2-x}Se$ species. For dual-channel detection, two distinct protocols were employed. For the CA-like activity assay, 50 μL of ZnSe clusters and 50 μL of Cu^{2+} (varying from 0.54 to 1.2 mM) were incubated in 1140 μL of Tris-HCl buffer (pH 9). Following the addition of 40 μL of p-NPA (10 mM) and a 30-minute reaction period, a 500 μL aliquot was diluted in 2.5 mL of H_2O. The absorbance was measured at 400 nm. Simultaneously, the peroxidase-like activity was evaluated in a pH 4.0 NaAc-Hac buffer (840 μL). The reaction mixture contained 40 μL of ZnSe clusters, 40 μL of Cu^{2+}, 40 μL of 30% H_2O_2, and 40 μL of TMB (5 mM). After a 30-minute incubation, the absorbance at 652 nm was recorded to quantify the Cu^{2+} concentration.

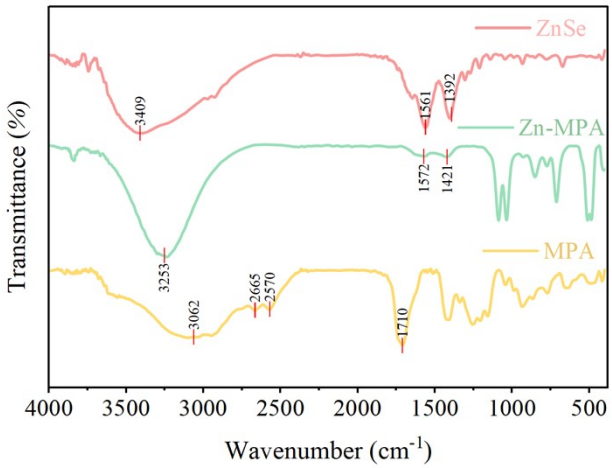

Figure S1. FT-IR spectra of ZnSe cluster, Zn-MPA complex, and MPA. Upon comparing the spectra of MPA and the Zn-MPA complex, the peak at 1710 cm^{-1} attributed to the carboxyl C=O stretching vibration of MPA shifts to 1561 cm^{-1} and 1392 cm^{-1}, which can be ascribed to coordination between Zn^{+2} and the carboxylate group. Further comparison between the Zn-MPA complex and the ZnSe cluster reveals that the peaks at 1421 cm^{-1} and 1572 cm^{-1} further shift to 1392 cm^{-1} and 1561 cm^{-1}, respectively, suggesting the formation of ZnSe clusters. Additionally, the broad absorption bands around 3410 cm^{-1} are associated with O–H stretching vibrations.

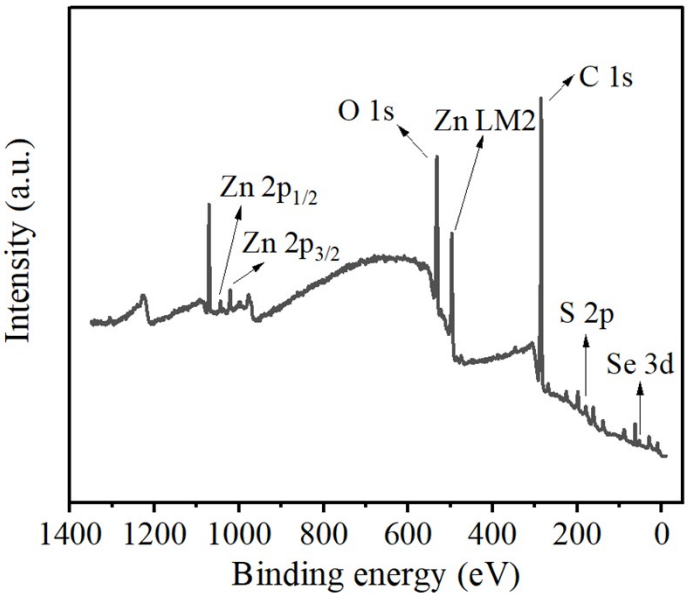

Figure S2. XPS survey spectra of ZnSe cluster.

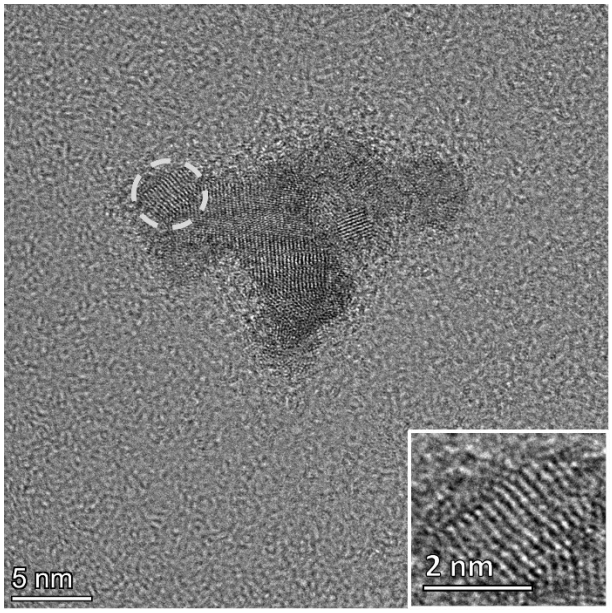

Figure S3. TEM spectra of ZnSe cluster.

Table S1. Catalytic activity parameters of ZnSe cluster and other CA-like catalysts.

Material	K_m (mM)	V_{max} (mM/min)	Refs
ZnTaz-1	4.30	0.014	[1]
ZIF-90	7.657	0.029	[2]
DW-CAB	6.17	0.230	[3]
ZC-HNPs	3.37	0.008	[4]
ZnAC	4.071	0.220	[5]
CA	154	102	[6]
ZnSe	3.55	15.04	This work

References

1. S. Liang, X.-L. Wu, M.-H. Zong and W.-Y. Lou, *Chemical Engineering Journal*, 2020, **398**, 125530.
2. C. Fan, Y. Tang, H. Wang, Y. Huang, F. Xu, Y. Yang, Y. Huang, W. Rong and Y. Lin, *Nanoscale*, 2022, **14**, 7985-7990.
3. L. Li, W. Xu, Z. Wu, W. Geng, S. Li, S. Sun, M. Wang, C. Cheng and C. Zhao, *Small*, 2024, **20**, 2307537.
4. B. Fan, Y. Zhang and Y. Lv, *Matter*, 2023, **6**, 4245-4260.
5. J. Liu, S. Sun, R. Lv, S. Lin, Y. A. Golubev, K. Wang, R. Cao and Y. Zeng, *Separation and Purification Technology*, 2025, **354**, 128979.
6. Ana M. Pablo-Sainz-Ezquerra, M. Rubio-Huertas, E. T. Tunca, P. W. Thulstrup and L. Hosta-Rigau, *Mater. Today Bio*, 2025, **35**, 102406.