Supporting Information for

Oxidation activity modulation of single atom Ce-N-C nanozyme enable time-resolved sensor to detect Fe^{3+} and Cr^{6+}

Guangchun Song [a‡], Qi Zhang [a‡], Shuang Liang [a], Yin Yao [a], Menglin Feng [a], Zainabu Majid [a], Xiaoyun He [a], Kunlun Huang [a, b], Jin-Cheng Li [c] and Nan Cheng [a*]

a. Beijing Laboratory for Food Quality and Safety, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China

b. Key Laboratory of Safety Assessment of Genetically Modified Organism (Food Safety), Ministry of Agriculture, Beijing 100083, China

c. Faculty of Chemical Engineering, Yunnan Provincial Key Laboratory of Energy Saving in Phosphorus, Chemical Engineering and New Phosphorus Materials, Kunming University of Science and Technology, Kunming 650000, China

‡ These authors contributed equally to this work.

** Corresponding author*

Email: chengnanFSNE@cau.edu.cn

1. *Characteristic of SACe-N-C*

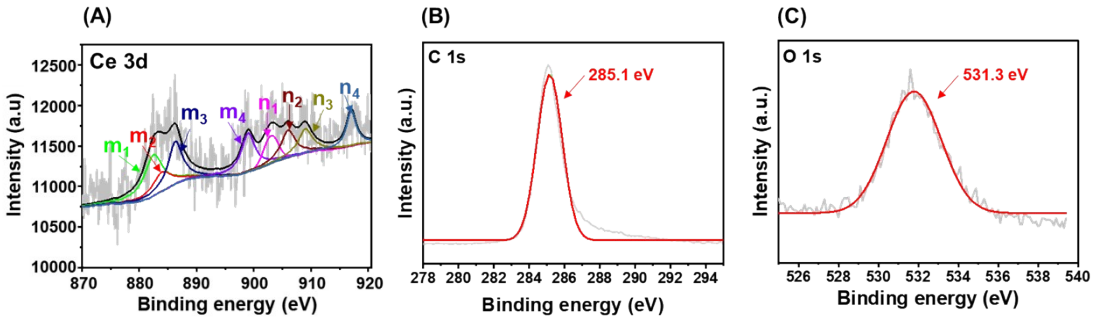

Fig. S1 XPS (A)Ce 3d, (B)C 1s and (C)O 1s spectra of SACe-N-C.

Fig. S1A showed the XPS Ce 3d spectra of SACe-N-C nanozyme. Eight peaks were fitted according to their binding energies. Among them, n_2 and m_2 were ascribed to Ce^{3+} while the others were for Ce^{4+}. XPS C1s spectra showed that the C binding energy of SACe-N-C was 285.1 eV (Fig. S1B). At the same time, XPS O1s spectra showed that the O binding energy of SACe-N-C was 531.3 eV (Fig. S1C).

2. *Optimization and evaluation of experimental conditions*

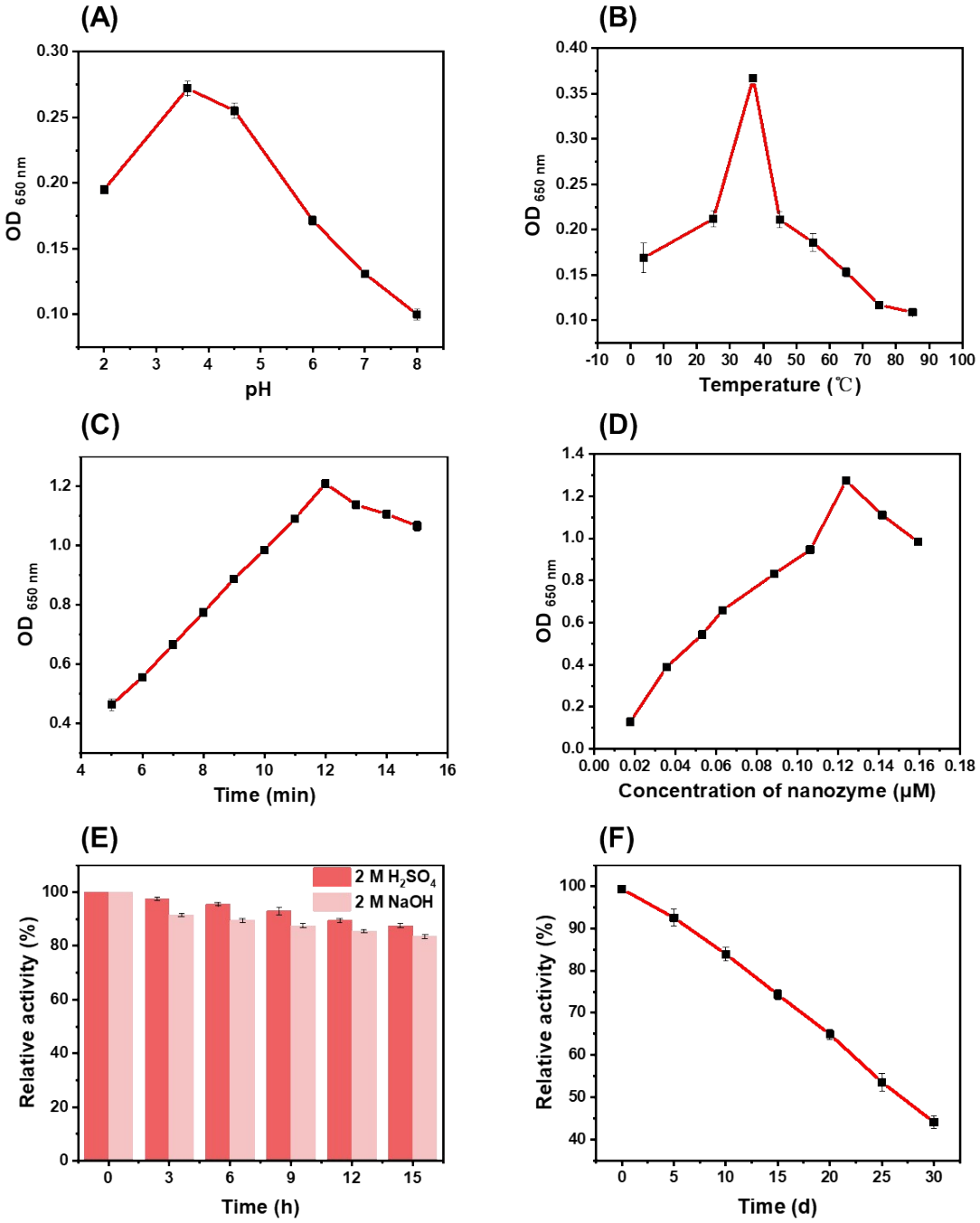

Fig. S2 Optimization of reaction conditions for OXD-like activity of the SACe-N-C nanozyme: (A) pH; (B) temperature; (C) Time; (D) the concentration of SACe-N-C nanozyme; (E) relative activity at 2 M H_2SO_4 and NaOH; (F) relative activity at 45℃.

3. Optimization and evaluation of experimental conditions

The calculation process is as follows:

Use the following formula for SA calculation:

$$b(nanozyme) = v/((\varepsilon \cdot l)) \cdot ((\triangle A)/(\triangle t))$$

Where *b(nanozyme)* represents the catalytic activity of a unit nanozyme which is defined as the number of nanozymes catalyzing to produce 1 μmol product per minute at 37℃; v is the total volume of the reaction system (μL); ε is the path length (cm) of

light propagation in the colorimetric plate which is called the molar absorption coefficient. The molar absorption coefficient of TMB at 652 nm is 39,000 M^{-1} cm^{-1} (OPD: $\varepsilon_{417\ nm}$ = 16700 M^{-1} cm^{-1}; ABTS: $\varepsilon_{420\ nm}$ = 36000 M^{-1} cm^{-1}) is the path length (cm) of light propagation in the colorimeter. *A* is the absorbance after subtracting the blank value; *ΔA/Δt* is the initial change rate of 652 nm/min absorbance.

$$a(nanozyme) = (b(nanozyme))/([m])$$

a(nanozyme) is SA (U mg^{-1}) expressed per milligram nanozyme; $[m]$ is the weight of nanozyme (mg) for each test. Due to *b(nanozyme)* catalytic activity is linearly related to its mass, by drawing the relation schema between *b(nanozyme)* and $[m]$, the slope of the line is the measured SA value.

Use the following formula for K_m, v_{max} and k_{cat} calculation:

$$v = (v(max)\cdot[s])/((Km + [s])\ )$$

v is the initial reaction rate, and $v(max)$ is the maximum reaction rate at the concentration of the saturated matrix, $[s]$ is the substrate concentration, K_m defined as half of the substrate concentration at the maximum rate is the Miltonian constant, reflecting the affinity of the nanozyme to the substrate.

$$k(cat) = v(max)/[E]$$

$k(cat)$ is the rate constant of the maximum number of substrate molecules transformation. $[E]$ is the concentration of nanozyme (M).

3. OXD-like activity parameters and comparison of SAzymes

Table S1 OXD-like activity parameters and comparison of SAzymes

Category	Species	[E]（M）	K_m（mM）	v_{max}（M s^{-1}）	k_{cat}（s^{-1}）	Stability (Maintain 80% activity)	References
SAzymes	FeN_5 SA/ CNF	5.37×10^{-7}	0.148	7.58×10^{-7}	0.708	/	[1]
	MnN_5 SA/ CNF	1.50×10^{-7}	0.253	4×10^{-7}	0.374	/	[1]
	CoN_5 SA/ CNF	0.31×10^{-7}	0.682	1.77×10^{-7}	0.174	/	[1]
	FeN_4 SA/ CNF	0.19×10^{-8}	0.143	4.5×10^{-8}	0.042	/	[1]
	NiN_5 SA/ CNF	3.6×10^{-13}	0.120	6×10^{-10}	0.0006	/	[1]
	CuN_5 SA/ CNF	2.35×10^{-13}	0.124	4.7×10^{-10}	0.0005	/	[1]
	Ce-N-C	8.85×10^{-8}	0.199	1.14×10^{-8}	0.129	15 h	This work
	Pt NPs	/	0.09	7×10^{-6}	/	3-4 h	[2]
	CeO_2	/	3.8	7×10^{-5}	/	/	[3]
	Ag@Ag_3PO4	/	2.09	/	/	0.06 h	[4]
	Pt nanoclusters	/	0.63	2.7×10^{-6}	/	2 h	[5]
	Ir NPs	1.72×10^{-10}	0.28	1.37×10^{-7}	/	/	[6]
	Au@Pt	5.0×10^{-12}	0.013	2.5×10^{-10}	500	Above 30 min	[7]
	$NiCo_2O_4$ Ms	20μg/ mL	0.127	9.99×10^{-9}	/	Above 2h	[8]
	MSN-Au NPs		0.2248	1.187×10^{-7}	/	Above 4 months	[9]
	FeCo NPs@PNC	2 mg/mL	0.091	9.39×10^{-8}	/	/	[10]

	$CeVO_4$	0.0985	3.94×10^{-8}	/	/	[11]

4. Detection results of Fe^{3+} and Cr^{6+} in real samples

Table S2 Detection results of Fe^{3+} and Cr^{6+}

metal ion	Real samples	The adding standard amount（μg/mL）	The amount of detection（μg/mL）	ICP-MS detection（μg/mL）	Recovery（%）	RSD（% n=3）
Fe^{3+}	Drinking water	0.5	0.71	0.71	101.43	1.43
	Tap water	0.5	0.72	0.71	113.24	2.71
	Wheat	0.5	0.65	0.66	100.41	0.62
	Peach	0.5	0.66	0.67	103.43	1.05
	Rose tea	0.5	0.64	0.64	88.66	0.98
	Black tea	0.5	0.62	0.63	103.78	2.11
	Celery	0.5	0.65	0.66	101.24	1.63
	Spinach	0.5	0.71	0.72	102.66	0.82
	Quenelle	0.5	0.65	0.68	101.22	1.17
	Frozen chicken	0.5	0.62	0.66	95.68	2.75
Cr^{6+}	Drinking water	0.5	0.42	0.44	85.49	3.01
	Tap water	0.5	0.44	0.45	89.42	2.78
	Wheat	0.5	0.53	0.53	106.41	4.34

Peach	0.5	0.43	0.44	86.27	1.76
Rose tea	0.5	0.48	0.47	97.28	2.12
Black tea	0.5	0.45	0.46	90.89	3.43
Celery	0.5	0.45	0.48	91.22	0.97
Spinach	0.5	0.55	0.57	111.22	1.13
Quenelle	0.5	0.42	0.45	84.61	1.59
Frozen chicken	0.5	0.47	0.50	95.89	2.92

63

Refrences

1 Huang, L.; Chen, J. X.; Gan, L. F.; Wang, J.; Dong, S. J., Single-atom nanozymes. *Sci Adv* 2019, **5 (5)**, eaav5490-5498.

2 You, J. G.; Liu, Y. W.; Lu, C. Y.; Tseng, W. L.; Yu, C. J., Colorimetric assay of heparin in plasma based on the inhibition of oxidase-like activity of citrate-capped platinum nanoparticles. *Biosensors and Bioelectronics* 2017, **92**, 442-448.

3 Asati, A.; Santra, S.; Kaittanis, C.; Nath, S.; Perez, J. M., Oxidase-like activity of polymer-coated cerium oxide nanoparticles. *Angew Chem Int Ed Engl* 2009, **48 (13)**, 2308-2312.

4 Chai, D. F.; Ma, Z.; Qiu, Y.; Lv, Y. G.; Liu, H.; Song, C. Y.; Gao, G. G., Oxidase-like mimic of Ag@Ag3PO4 microcubes as a smart probe for ultrasensitive and selective Hg(2+) detection. *Dalton transactions (Cambridge, England: 2003)* 2016, **45 (7)**, 3048-3054.

5 Yu, C. J.; Chen, T. H.; Jiang, J. Y.; Tseng, W. L., Lysozyme-directed synthesis of platinum nanoclusters as a mimic oxidase. *Nanoscale* 2014, **6 (16)**, 9618-9624.

6 Cui, M.; Zhao, Y.; Wang, C.; Song, Q., The oxidase-like activity of iridium nanoparticles, and their application to colorimetric determination of dissolved oxygen. *Microchimica Acta* 2017, **184 (9)**, 3113-3119.

7 He, W.; Liu, Y.; Yuan, J.; Yin, J. J.; Wu, X.; Hu, X.; Zhang, K.; Liu, J.; Chen, C.; Ji, Y.; Guo, Y., Au@Pt nanostructures as oxidase and peroxidase mimetics for use in immunoassays. *Biomaterials* 2011, **32 (4)**, 1139-1147.

8 Su, L.; Dong, W.; Wu, C.; Gong, Y.; Zhang, Y.; Li, L.; Mao, G.; Feng, S., The peroxidase and oxidase-like activity of NiCo(2)O(4) mesoporous spheres: Mechanistic understanding and colorimetric biosensing. *Anal Chim Acta* 2017, **951**, 124-132.

9 Tao, Y.; Ju, E.; Ren, J.; Qu, X., Bifunctionalized mesoporous silica-supported gold nanoparticles: intrinsic oxidase and peroxidase catalytic activities for antibacterial applications. *Advanced materials (Deerfield Beach, Fla.)* 2015, **27 (6)**, 1097-1104.

10 Wu, T.; Ma, Z.; Li, P.; Liu, M.; Liu, X.; Li, H.; Zhang, Y.; Yao, S., Colorimetric detection of ascorbic acid and alkaline phosphatase activity based on the novel oxidase mimetic of Fe-Co bimetallic alloy encapsulated porous carbon nanocages. *Talanta* 2019, **202**, 354-361.

11 Yang, H., Zha, J., Zhang, P., et al. Fabrication of CeVO4 as nanozyme for facile colorimetric discrimination of hydroquinone from resorcinol and catechol. *Sensors & Actuators B Chemical*, 2017, **247**, 469-478.