Electronic Supporting Information

1 Experimental

1.1 Materials and reagents

Okadaic acid (OA), Domoic acid (DA), mefenamic acid (MEF), Tetrodotoxin (TTX), Tetrabromobisphenol A (TBBPA), Keyhole limpet hemocyanin (KLH), Bovine serum albumin (BSA), Ovalbumin (OVA) , horseradish peroxidase (HRP) conjugated goat anti-mouse IgG antibody were obtained from Sigma-Aldrich, USA. Sodium citrate tribasic dehydrate (Shanghai Titan chem. Co., Ltd., Shanghai, China), L-ascorbic acid (Aladdin industrial corporation, Shanghai, China), chloroauricacidtetra hydrate ($HAuCl_4 \cdot 4H_2O$, 48-50% Aubasis) and potassium tetrachloroplatinate (Shanghai Macklin Biochemical Co., Ltd.), 3,3',5,5'-tetramethylbenzidine (TMB, Aladdin industrial corporation, Shanghai, China), and polystyrene microplates (Costar, Corning Inc., NewYork, USA) were used without pretreatment.

Coating buffer: 50 mM sodium carbonate, pH 9.6; Blocking buffer: 10 mM PBS including 1wt% BSA, pH 7.4; Washing buffer: 10 mM PBS including 0.5 wt% Tween 20, pH 7.4.

1.2 Methods

1.2.1 Test of hybridoma cells

The binding activities of hybridoma cells were tested by ic-ELISA. A 96-well plate was coated with OA-BSA antigen (5 μg/mL, 100 μl/well) in 0.05 M carbonate/bicarbonate buffer (pH 9.60) overnight at 4°C. After the coating procedure, the plates were washed 3 times with phosphate buffer solution (1×PBS), and then blocked with PBS-milk solution (PBSM, the content of milk was 5%, 200 μL/well) at 37°C for 2 h.

The wells were washed three times with tween 20 (0.1%)-phosphate buffer and 1×PBS. The cell supernatant was diluted in PBSM and incubated at 37°C for 1 h. After washing, HRP-labeled goat anti-mouse IgG (1:8000 diluted by 5% PBSM, 100μL/well) was added and incubated at 37°C for 1 h. Clean the well and a 100μL substrate solution was added into each well at 37°C for 10 min. Finally, the reaction was stopped by 2 M H_2SO_4 (50 μL/well), and the value of optical density (OD) was measured at 450 nm.

1.2.2 Affinity determination of McAb

According to the method of Beatty (Beatty et al, 1987) and Dai (Dai, et al, 2003), the affinity constant K_{aff} was determinated by iELISA. This method for the estimation of K_{aff} is simple, rapid, and reliable. Simply, OA-BSA antigens with different concentrations were coated in the enzyme-labeled wells, and blocked with 5% PBSM buffer solution. The anti-OA monoclonal antibody was doubling diluted and prepared

in different concentrations, then added to the enzyme-labeled wells and incubated at 37°C for 1 h.

Accordance to the following formula, the affinity constant (K_{aff}) was calculated.

$K_{aff} = (n-1)/\{2(n\,[Ab_2]-[Ab_1])\}$,

Where [Ag] was concentration of coated antigen, $[Ab_1]$ and $[Ab_2]$ represent the different McAb concentrations rrequired to achieved 50% of the OD_{max} at two different concentrations of coated antigen ($[Ag_1] = n\,[Ag_2]$), and $n = [Ag_1] / [Ag_2]$.

References:

[1] J. D. Beatty, B. G. Beatty, W. G. Vlahos. J Immunol Methods. 100(1987) 173.
[2] H. Dai, H. Gao, X. Zhao, L. Dai, X. Zhang, N. Xiao,R. Zhao, S. M. Hemmingsen, J Immunol Methods. 279 (2003) 267.

1.2.3 Specificity determination of McAb

According to the ref. [Talanta, 2021, 228, 122215], the cross-reactivity of McAb against OA to domoic acid (DA), sea snake venoms (SN311), tetrodotoxin (TTX), and mixed interferences were determined by the icELISA. Here, SN311 at 10 ng/mL, DA at 300 ng/mL and TTX at 1.0 ng/mL were spiked in the reagent buffer. OA was spiked at 1 ,4 and 15 ng/mL. Interference from L-glutamic acid (usually present in shellfish tissue), mefenamic acid (MEF) and tetrabromobisphenol A (2,3-dibromopropyl) ether (TBBPA) was also tested. Mix Interf. was a mixture of L-glutamic acid, mefenamic acid (MEF) and tetrabromobisphenol A (2,3-dibromopropyl) ether (TBBPA) at 10 μg/mL per compound. The mixed solutions with different combinations were added to each well of OA-BSA coated plate. The plate was incubated at 37°C for 1 h.

1.2.4 Detection procedure of OA

The OA-BSA antigen was diluted with carbonate coating buffer according to the optimal antigen coating concentration, and injected into the high-binding 96-well microplate at 100μL each well and incubating overnight at 4°C. The microplate was then rinsed 3 times with washing buffer (200 μL perwell) and blocked with blocking buffer (200 μL perwell) with an incubation for 2 h at 4°C. the obtained OA-BSA antigen coated microplate was rinsed 3 times with washing buffer (200 μL perwell).

A 100 μL OA standard solution was mixed with Au@Pt-McAb at the ration of 1:1, and maintained at 37 °C for 1 h. Then the obtained solution was added into the microplate (100 μL perwell) and incubated at 37 °C for 1 h. After washing with washing buffer for 3 times (200 μL perwell each time), the horseradish peroxidase (HRP) conjugated goat anti-mouse IgG antibody Diluted in a certain proportion was added into the above microplate (100 μL perwell), and reacted at 37 °C for 60 min.

After washing for 3 times (200 μL perwell each time),TMB substrate (100 μL) was added into each well, and incubated for 15 min at 37 °C. Finally, the catalytic reaction was stopped by using H_2SO_4 (50 μL, 2M). The absorbance was recorded at 450 nm using a microplate reader (SYNERGYH1, BioTek.).

Supporting data:

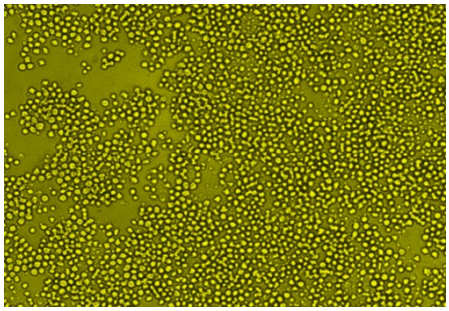

Fig. S1 The picture of Hybridoma cells

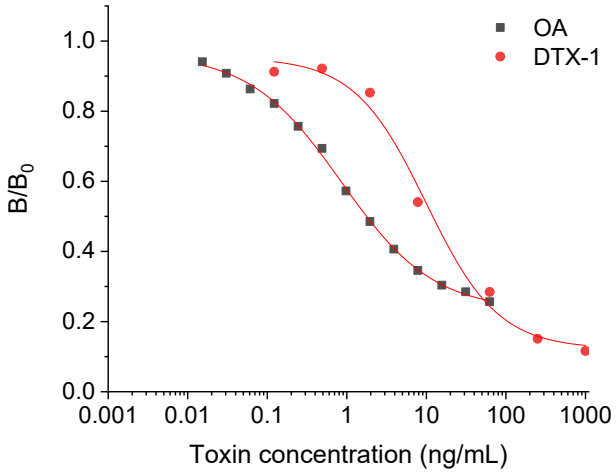

Fig. S2 Binding curve for OA and DTX-1 detection of ic-ELISA with Au@Pt-McAb composites
DTX-1: R^2=0.98, IC_{50}=9.95 ng mL^{-1}; OA: R^2=0.99, IC_{50}=0.87 ng mL^{-1}.
Cross-reactivity = (([IC_{50}] for OA)/([IC_{50}] for DTX))*100% = 8.7%

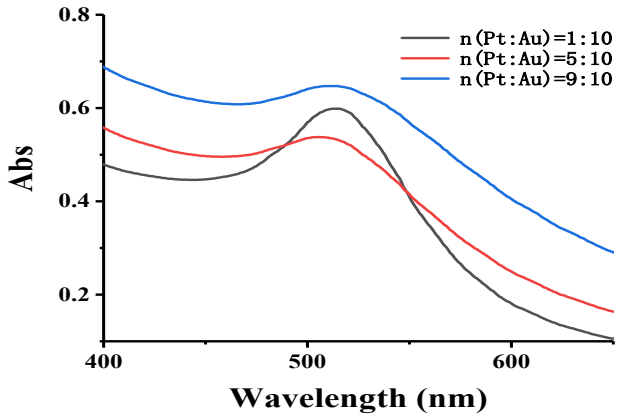

Fig. S3 UV-vis spectra scanning of Au@Pt NPs with different mole ratios

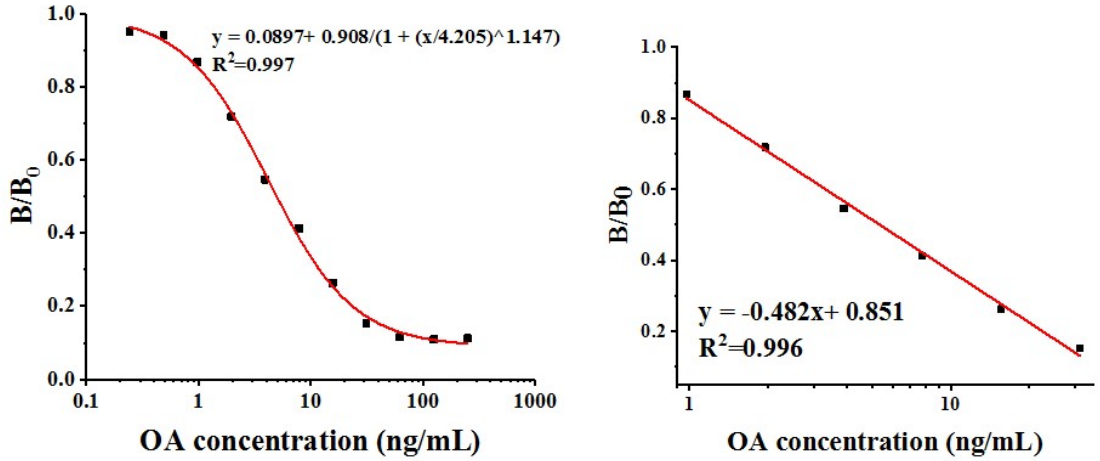

Fig. S4 The calibration curve for OA detection of ic-ELISA with HRP .

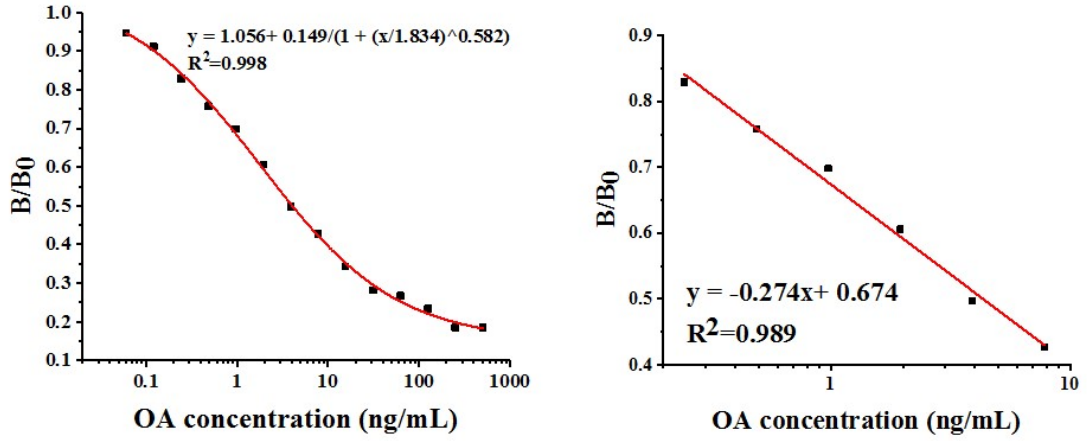

Fig. S5 The calibration curve for OA detection of ic-ELISA with AuNPs@HRP

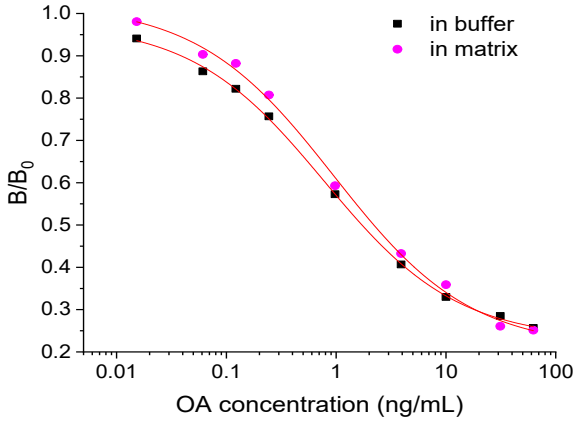

Fig. S6 ic-ELISA for OA in buffer or in matrix

Table S1 Post-hoc analyses of a one-way ANOVA for catalytic activity of Au@Pt-McAb

Tests for between-subject effects

Dependent variable: catalytic activity indicated as OD450nm

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	0.429[a]	2	0.214	6.907	0.028
Intercept	23.792	1	23.792	766.192	0.000
Au@Pt-McAb composites	0.429	2	0.214	6.907	0.028
Error	0.186	6	0.031		
Total	24.407	9			
Corrected total	0.615	8			

a. R square = 0.697（Adjusted R square = 0.596）

F =6.907，P（Sig.=0.028）< 0.05, that is significant at the 0.05 level.

Multiple comparisons

Dependent variable: catalytic activity indicated as OD450nm

	(I) group-i	(J) group-j	mean difference (I-J)	standard error	Sig.	95% confidence interval lower limit	95% confidence interval upper limit
LSD	1	2	-0.39800*	0.143879	0.033	-0.75006	-0.04594
		3	0.11033	0.143879	0.472	-0.24173	0.46239
	2	1	0.39800*	0.143879	0.033	0.04594	0.75006
		3	0.50833*	0.143879	0.012	0.15627	0.86039

	3	1	-0.11033	0.143879	0.472	-0.46239	0.24173
		2	-0.50833*	0.143879	0.012	-0.86039	-0.15627

Based on the observed mean. Error term is mean squared (error) = 0.031。

*. Differences in means are significant at the 0.05 level.

Table S2 The optimal matching concentrations of coating and Au@Pt NPs-McAb

Dilution factors of Au@Pt NPs-McAb	Dilution factors of OA-BSA				
	1/400	1/800	1/1600	1/3200	1/6400
1/10	2.342	2.609	2.474	2.400	2.290
1/20	2.324	2.454	2.363	2.362	2.186
1/40	2.282	2.343	2.227	2.336	2.056
1/80	2.168	2.187	2.089	1.083	1.866
1/160	1.722	1.777	1.765	1.713	1.480
1/320	1.504	1.439	1.202	1.209	0.968
1/640	1.195	1.037	0.923	0.816	0.627
1/1280	0.813	0.735	0.707	0.569	0.440

Table S3 The comparison with ELISA methods reported for OA detection

Detection methods	IC_{50}（ng/mL）	IC_{10}（ng/mL）	Ref.
ic-ELISA	0.33	0.06	[8]
ic-ELISA	4.40	0.45	[9]
ic-ELISA	0.98	0.18	[37]
c-ELISA	2.07	0.6	[37]

ic-ELISA	22.4	7.4	[38]
AuNPs synergistic ic-ELISA	1.83	0.12	This work
Pt@AuNPs synergistic ic-ELISA	0.87	0.04	This work

References:

[8] A. Sassolas, G. Catanante, A. Hayat, L. D.Stewart, C. T.Elliott, J. LouisMarty, Food Control, 2013, 30,144.

[9] S. Y. Lu, Y. Zhou, Y. S. Li, C. Lin, X. M. Meng, D. M. Yan, Z. H. Li, S. Y. Yu, Z. S. Liu, H. L. Ren. Environ Sci Pollut Res Int, 2011, 19(7):2619

[37] S. Y. Lu, Y. Zhou, Y. S. Li, H. L. Ren, F.Z. Huo, Y. Wang, Z.S. Liu, G. Yu. Chin. J. Vet. Sci, 2007, 27: 336.

[38] M.L. Berre, M. Kilcoyne, M. Kane, Toxicon,2015, 103,169.