Electronic Supplementary Information

A novel, colorimetric method for biogenic amine detection based on arylalkylamine N-acetyltransferase

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Reagents and materials. The competent *Escherichia coli* BL21 (DE3) strains were purchased from TransGen (Beijing, China). J774A.1 murine macrophage cell line was purchased from China Center for Type Culture Collection (Wuhan, China). Fetal bovine serum (FBS) and Dulbecco's Modified Eagle's Medium were purchased from Life Technologies (AB & Invitrogen, Carlsbad, USA). The mouse histamine ELISA kit was purchased from Blue Gene (Shanghai, China). The pET28a plasmid was purchased from Novagen (Gibbstown, NJ). Chemical reagents, including 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB), acetyl coenzyme A (AcCoA), amikacin (AMK), kanamycin (KAN), neomucin (NEO), imidazole, trichloroacetic acid, histamine (HI), tyramine (TA), phenylethylamine (PEA), glycine(G), valine (V), leucine (L), phenylalanine (F), proline (P), tyrosine (T), arginine(R), tryptophan (W), lysine(K), histidine (H), tert-butylamin (t-BA), n-propylamine (t-PA), isopropyl-β-D-thiogalactoside (IPTG), and lipopolysaccharide (LPS) were purchased from Sigma-Aldrich (Milwaukee, WI). EcoRI and HindIII were purchased from TaKaRa Biotechnology Co., Ltd. (Dalian, China). Nickel-ni-trilotriacetic acid columns were purchased from Merck & Co., Inc. (Darmstadt, Germany). Amicon Ultra-430K cutoff centrifugal devices were purchased from Millipore Corp. (Billerica, MA, USA).

Instrumentation. UV-Vis absorption was measured by a microplate reader Synergy™ Mx (Bio-Tek Instruments, Winooski, VT) using a clear flat bottom 96-well microplate (Greiner, Germany).

Overproduction and purification of arylalkylamine N-acetyltransferase (aaNAT). The *aanat* gene was synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China), and incorporated into a pET28a vector using EcoRI and HindIII. The nucleic acid sequence of interest

was optimized in order to ensure that the codon bias was consistent with that from *Escherichia coli* BL21 (DE3) strains. The gene sequences are shown in Fig. S1.

Fig. S1 Optimization of the *aanat* gene. The letters of *aa* represent the gene of aanat and opt*aa* is the optimized sequence.

Expression of the *aanat* gene from plasmid pET28a was achieved in *Escherichia coli* BL21 (DE3) strain. The host strains were first inoculated in 5 mL Luria Bertani (LB) medium at 37 °C for 12 hours, and then expanded in 50 mL LB broth with 50 μg/mL kanamycin in case of foreign invasion. When the optical density (OD_{600}) of the culture reached 0.6, IPTG with a final concentration of 0.7 mM was added to the medium to induce protein expression and cells were grown for an additional 12 h at 20 °C. Bacterial cells were harvested by centrifugation at 5, 000 rpm for 30 min, and then the cell pellet was rinsed in phosphate buffered saline (PBS) buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4). The cells were disrupted by sonication, and cell debris was removed by centrifugation at 5,000 rpm at 4 °C for 30 min. The resultant supernatant flowed through the his-binding affinity chromatography resin (Qiagen,

Germany) and aaNAT bound to the resin due to the his-tag on the N-terminal of the protein. To remove other proteins, the resin was washed with 20 mM, 40 mM, and 60 mM imidazole dissolved in 50 mM NaH_2PO_4 with 300 mM NaCl (pH 8.0). Then, the protein of interest was eluted with 250 mM imidazole which was also dissolved in NaH_2PO_4 with 300 mM NaCl (pH 8.0). The purified aaNAT was dialyzed against 50 mM PBS (pH 7.4) overnight at 4 °C for the removal of imidazole and then was concentrated using an Amicon Ultra-430K cutoff centrifugal device. The concentration of aaNAT was determined by the bicinchoninic acid (BCA) method using PBS buffer as the control.

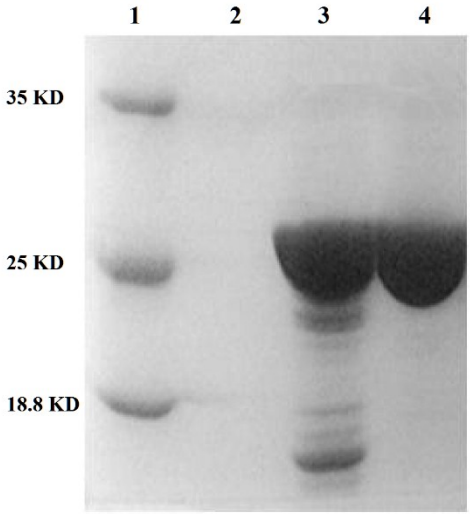

Fig. S2 SDS-PAGE image of aaNAT. Lane 1: protein marker; Lane 2: blank control; Lane 3: aaNAT supernatant; Lane 4: aaNAT after purification.

Biogenic amine detection procedures. The reaction mixtures for biogenic amine detection were prepared separately as part A and part B solutions. The part A solution consisted of AcCoA (1000 μM), biogenic amines, and HEPES buffer (50 mM, pH 7.5) in a volume of 50 μL. The part B solution consisted of aaNAT protein (0.5 μM), DTNB (2 mM) in a volume of 50 μL. Frist, the part A and part B solutions were incubated at 25 °C for 5 minutes. Subsequently, part A and part B were mixed together and incubated at 25 °C for 20 min. The reaction was stopped by heating at 95 °C for 2 minutes. Finally, the mixture was transferred to a 96-well plate and measured by a UV-vis microplate reader. In the real-time monitoring of the enzymatic reaction was acquired by detecting

at 412 nm every 30 s for 20 min at 25 °C using a UV-vis microplate reader. The data were fitted to a Michaelis-Menten curve to determine K_m and v_{max} values using GraphPad Prism 5. All reactions were performed at least in triplicate.

Food sample analysis. In order to test the applicability of the proposed method in food sample, white wine and milk were chosen. White wine sample was prepared by distilling to remove alcohol, and the left was collected for the following use. Milk sample was prepared according to a previously reported method.[1] Briefly, 2 mL of 20% trichloroacetic acid were added to 10 mL milk sample for protein coagulation, and protein debris was removed by centrifugation at 10,000 rpm for 30 min. Then, the supernatant was transferred to a 50 mL centrifuge tube. In this way, the interfering substances in the milk were removed. Samples showing the absence of biogenic amines of interest were used as blanks. In the spike-in experiments, a specified biogenic amine with known concentrations were added to the pretreated white wine and milk sample, and detected following the above procedures. Recovery data were expressed as mean ± SD.

Macrophage J774A.1 sample analysis. J774A.1 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 ºC under 5 % CO_2 humidified air. Cells were plated in a 12-well plates at a density of 1×10^5 cells/well and incubated overnight before stimulated with LPS (1 μg/ml) for 18 hours, 24 hours, 36 hours, 48 hours respectively. Then the cell culture supernatant was collected and transferred into a 2 mL centrifuge tube before detection.

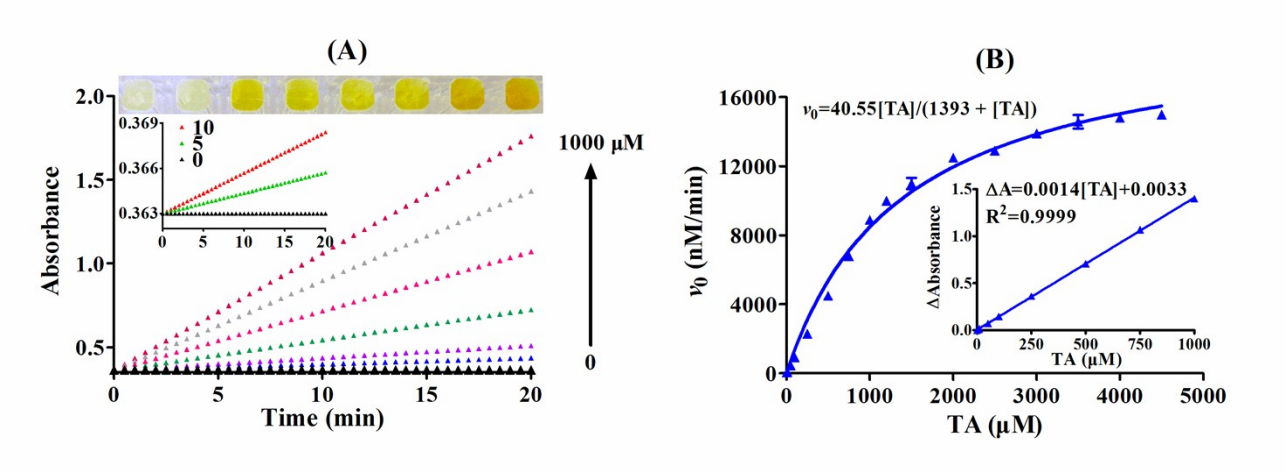

Fig. S3 Progress curves of absorbance at 412 nm vs time for aaNAT (0.5 µM) in the presence of tyramine (TA) at different concentrations (0, 5, 10, 50, 100, 250, 500, 750, and 1000 µM). Inset: magnification of the plot at the concentrations of 1, 5, and 10 µM. (B) Plot of the initial velocities for an increasing concentration of tyramine in the presence of aaNAT (0.5 µM) and DTNB (2 mM). Inset: Linear plot of the change of absorbance after 20 min vs tyramine concentration in the range of 0-1000 µM.

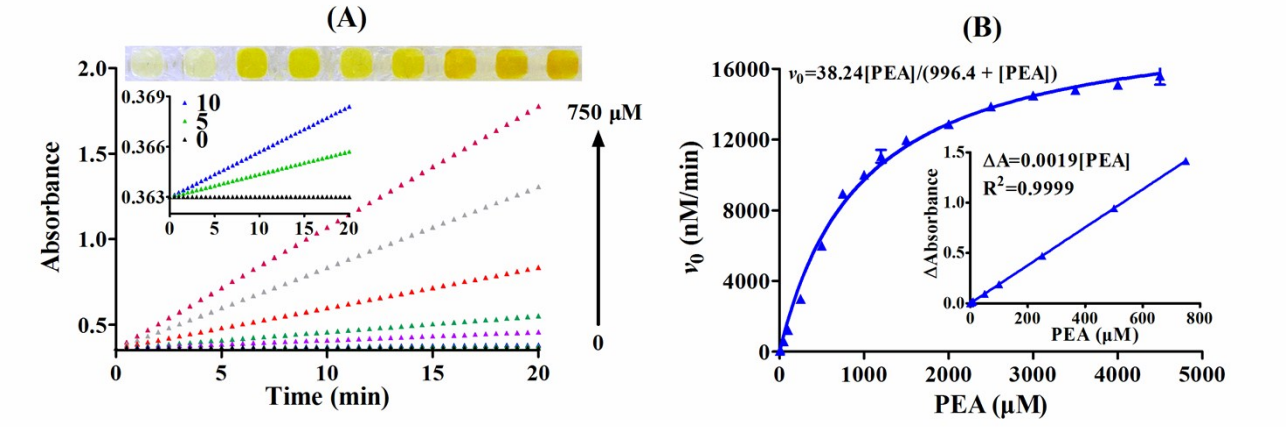

Fig. S4 (A) Progress curves of absorbance at 412 nm vs time for aaNAT (0.5 µM) in the presence of phenylethylamine (PEA) at different concentrations (0, 5, 10, 50, 100, 250, 500, and 750 µM). Inset: magnification of the plot at the concentrations of 1, 5, and 10 µM. (B) Plot of the initial velocities for an increasing concentration of phenylethylamine in the presence of aaNAT (0.5 µM) and DTNB (2 mM). Inset: Linear plot of the change of absorbance after 20 min vs phenylethylamine concentration in the range of 0-800 µM.

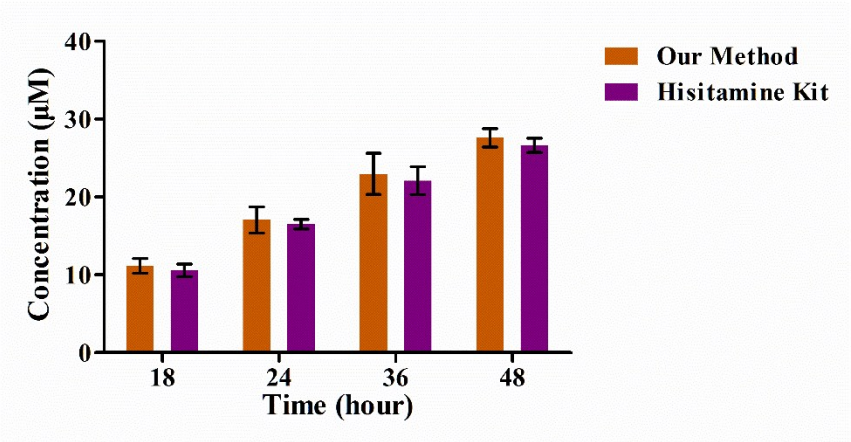

Fig. S5 Practical application test of histamine detection in cell supernatant of Macrophage J774A.1 stimulated by LPS for 18, 24, 36, 48 hours. Bars represent the amount of histamine using our proposed method (brown bars) and the mouse histamine ELISA kit (purple bars), respectively.

Table S1. Determination of histamine in food samples

Sample	Detected(μM)	Added (μM)	Found (μM)	Recovery (%)	RSD (%)
White wine	0	5.00	5.87±0.34	117.40	5.77
		20.00	20.53±0.67	102.65	3.26
Milk	0	5.00	5.95±0.44	119.00	7.48
		20.00	20.48±0.63	102.4	2.94

Table S2. Determination of tyramine in food samples

Sample	Detected (μM)	Added (μM)	Found (μM)	Recovery (%)	RSD(%)
White wine	0	5.00	5.53±0.21	110.6	3.71
		20.00	20.17±0.28	100.85	1.37
Milk	0	5.00	5.19±0.36	103.80	7.01
		20.00	19.41±0.39	97.05	1.99

Table S3. Determination of phenethylamine in food samples

Sample	Detected (μM)	Added (μM)	Found (μM)	Recovery (%)	RSD (%)
White wine	0	5.00	5.18±0.12	103.60	2.41
		20.00	19.8±0.64	99.00	3.23
Milk	0	5.00	5.05±0.24	101.00	4.82
		20.00	19.66±0.24	98.30	1.22

Reference

1 F. Liu, Y. Gao and Y. Zhao, *Anal. Methods*, 2014, **6**, 4661.