Electronic Supporting Information (ESI)

Spectrophotometric detection of tyrosinase activity based on boronic acid-functionalized gold nanoparticles

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Experimental sections

Materials and reagents

Tyrosinase, 16-mercaptohexadecanoic acid (MHDA), 3-aminophenylboronic acid (APBA), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich Co. (USA). Tyrosyl-tyrosine (Tyr-Tyr) and tyrosyl-glycyl-tyrosine (Tyr-Gly-Tyr) were obtained from ChinaPeptides Co., Ltd (China). Hydrogen tetrachloroaurate(III) trihydrate ($HAuCl_4 \cdot 3H_2O$) was procured from Shanghai July Chemical Co., Ltd (China). All other chemicals were of analytical grade. Ultrapure water (18.2 MΩ cm) was employed in all of the investigations.

Synthesis of gold nanoparticles (AuNPs)

AuNPs were synthesized through reduction of $HAuCl_4$ by citrate according to the literature[S1] with slight modification. Basically, a 100 mL of 0.01% (*w/v*) $HAuCl_4$ was added into a round-bottom flask, stirred to boil. Then 3.5 mL of 1% trisodium citrate (Citrate·3Na) was added rapidly into the boiling solution. The color of the solution gradually turned from colorless to wine red. The solution was stirred for another 30 min. The average size of the prepared AuNPs is 13 nm characterized by TEM, and the concentration of AuNPs was estimated to be 3.5 nM. The AuNPs solution was then centrifuged at 13,000 rpm for 20 min. The supernatant was removed and the precipitate was re-suspended into 1/5 volume of 1 mM phosphate buffer solution (pH7.0). Thus, the concentration of AuNPs was raised to 17.5 nM.

Preparation of the functionalized AuNPs

The sequential functionalization of AuNPs with MHDA and APBA was followed the reported procedure [S2] with modifications. AuNPs solution was degassed with nitrogen before use. Equal volumes of AuNPs solution and Tween 20 in buffer were gently mixed and allowed to stand for 20 min. Then MHDA was added and the mixture was allowed to stand for 4 h in darkness. The final concentration in the mixture: 1.5 nM

AuNPs, 2.0 mg mL^{-1} Tween 20, and 0.17 mM MHDA. Finally, the mixture was centrifuged and the precipitate was re-suspended in 10 mM HEPES buffer (pH7.2). The prepared MHDA/AuNPs was characterized by UV-visible spectroscopy. To further modify boronic acid group, APBA, EDC, and NHS were added to the MHDA/AuNPs solution to the final concentrations of 0.1, 0.8 and 2 mM, respectively. The mixture was allowed to stir for 3 h. Excess chemicals were removed by centrifugation and the prepared APBA/MHDA/AuNPs was re-suspended in 10 mM HEPES buffer (pH7.2) and characterized by UV-visible spectroscopy.

References

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S2 (a) K. Aslan and V. H. Pérez-Luna, *Langmuir*, 2002, **18**, 6059; (b) B. Basnar, J. Xu, D. Li, and I. Willner, *Langmuir*, 2007, **23**, 2293.

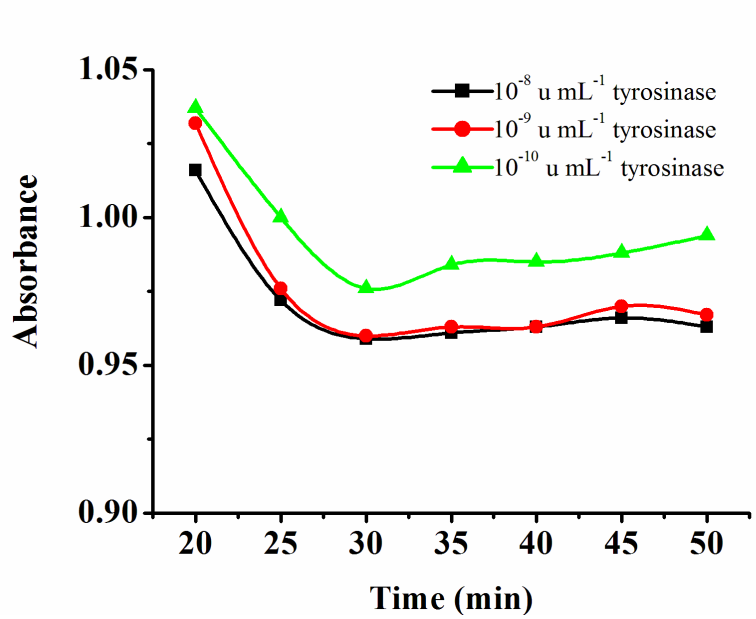

Fig. S1 The absorbance changes in UV-visible spectra of the mixture of the functionalized AuNPs and the enzymatic products with different incubation time.

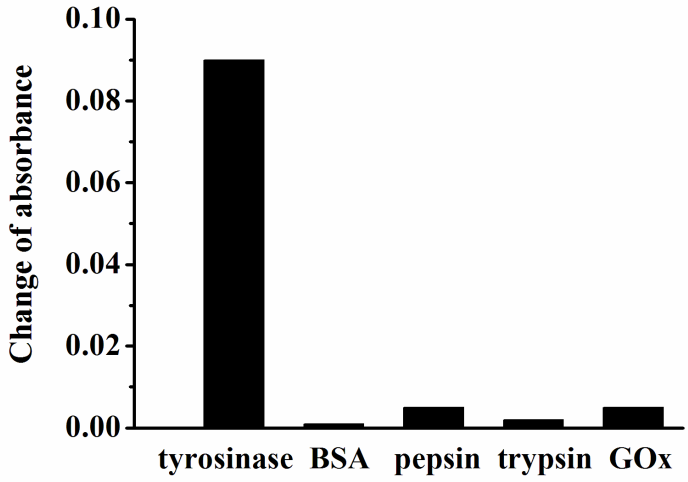

Fig. S2 The absorbance changes in UV-visible spectra of the mixture of the functionalized AuNPs and the reaction products with 1×10^{-8} u mL^{-1} tyrosinase, 100 μM bovine serum albumin (BSA), 1×10^{-6} u mL^{-1} pepsin, 1×10^{-6} u mL^{-1} trypsin, and 1×10^{-6} u mL^{-1} glucose oxidase (GOx).

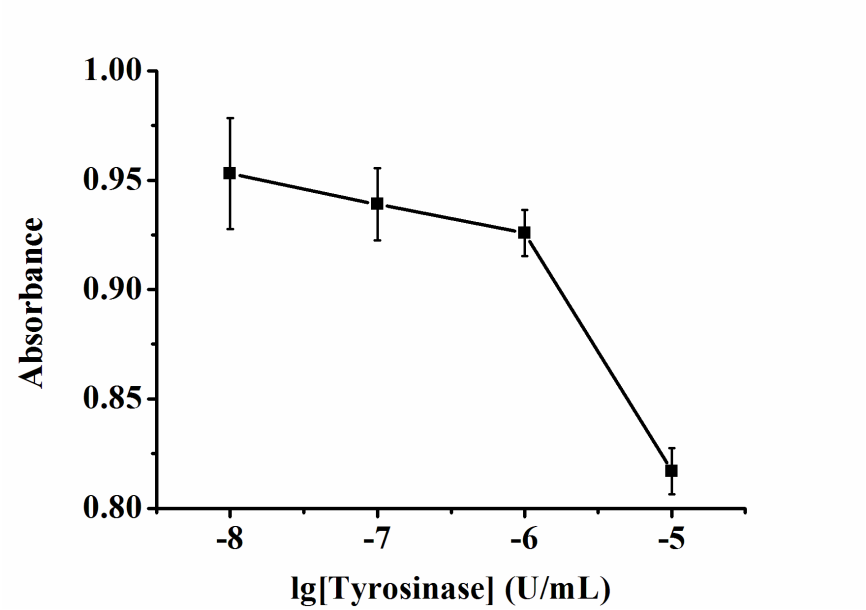

Fig. S3 The relationship between the absorbance at 530 nm in UV-visible spectra and the logarithm of the concentration of tyrosinase, by using Tyr-Gly-Tyr as the substrate.