Supplementary information

A novel enzyme-Immobilized flow cell used as end-column chemiluminescent detection interface in open-tubular capillary electrochromatography

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Table of contents

Derivatization of Gly with ABEI. Derivatization of Gly with ABEI was accomplished with a TEA-catalyzed method. In brief, 200 μl of 0.50 mM ABEI in methanol was added into an equal volume of 0.50 mM DSC in acetonitrile. The mixture was allowed to react at RT for 2 h. Then, 100 μl of sample in methanol and 20 μl of 0.15 M TEA in methanol were added into the above obtained ABEI-DSC solution. After vortex mixing, the mixture was allowed to react at RT for 2 h. The derivatized sample was then diluted for 10 folds with water prior to the sample loading.

Pretreatment of Saliva and Urine Samples. A 500-μl sample in a 3.0-ml centrifuge tube was mixed with 1.0 ml of acetonitrile and shaken vigorously for 2 min to precipitate proteins. After centrifuging at 10,000 rpm for 10 min, the supernatant was transferred into a 1.5-ml vial and dried with a gentle nitrogen stream. The residue was re-dissolved in 500 μl of methanol. The obtained samples were derivatized immediately with ABEI according to the above described protocol.

Synthesis of CD-GNPs. Two hundred microliter of *β*-CD-SH solution at 100 mg/ml was added into 20 ml of $HAuCl_4$ solution at 1.4 mM. After being stirred for 20 min at RT and added with 60 μl of NaBH4 solution at 10 mM, the mixture was vigorously stirred for 10 min at RT in darkness and for another 30 min, and left at RT overnight. The obtained solution in deep wine red was stored at 4 °C until use.

Evaluation of Activity of Immobilized Enzyme. Five microliter of HRP solution (1.0 μg/ml in 0.10 M PB, pH 7.4) was coated in one well of a polystyrene 96-well

plate (as control). The equal amount of HRP was chemically immobilized on an aldehyde-actived membrane, and the membrane adhered to the bottom of another well of the plate. The CL reagents, 40 μl of luminol solution at 0.1 mM and 40 μl of BPB solution at 1.0 μM were mixed and injected into each well. Finally, the CL signal was recorded with a microplate reader (Infinite 200 PRO, Tecan Group Ltd., Salzburg, Austria) after injection of 20 μl of H_2O_2 solution at 1.0 mM. Since the enzyme in the control well was immobilized by a physical adsorption, it was believed to remain most of its activity. Thus the comparison of the CL signals from the two wells can be utilized to evaluate the activity loss of the enzyme chemically immobilized on the aldehyde-actived membrane.

Fig. S1

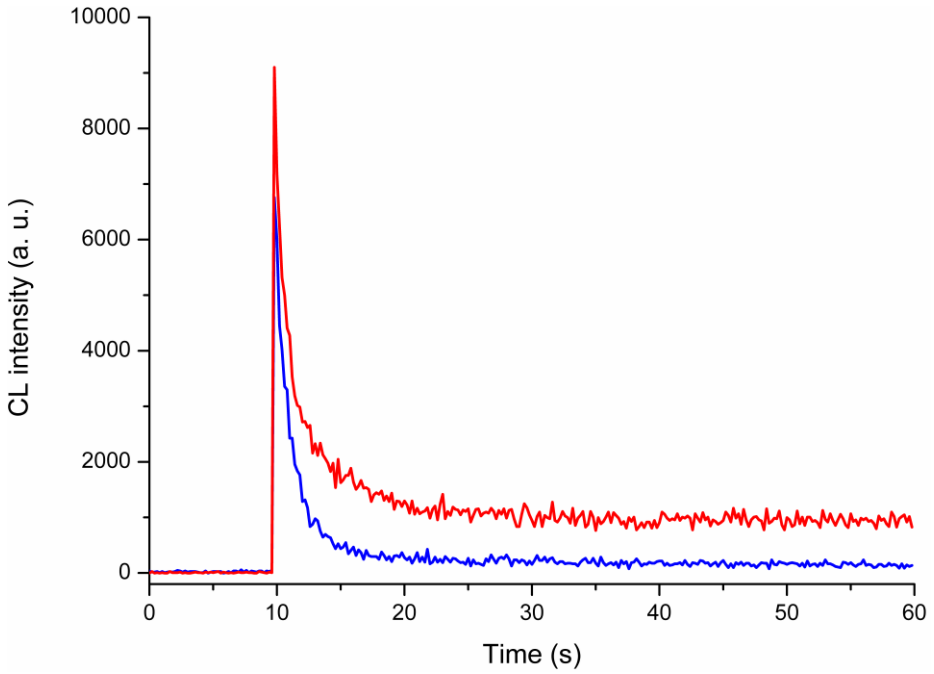

Fig. S1 CL responses from the reactions catalyzed by the enzyme immobilized on the aldehyde-actived membrane (blue) and coated in the well of the polystyrene plate (red). Luminol: 50 μM, BPB: 0.5 μM, H_2O_2:1.0 mM.

Fig. S2

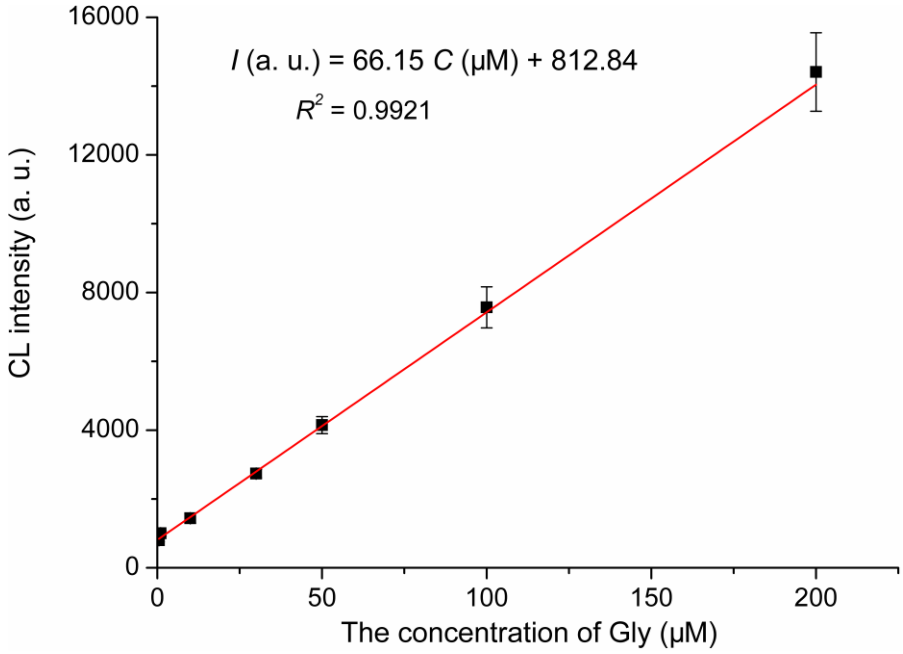

Fig. S2 CL intensity of Gly at the concentrations of 0.50, 1.0, 10, 30, 50, 100 and 200 μM, all conditions were the selected optimal conditions.