Supporting Information for

Single-cell-resolved Measurement of Enzyme Activity at a Tissue using Drop-on-demand Microkits

Rongcan Huang[1], Rong Jin[1], Dechen Jiang[1*], Hong-Yuan Chen[1]

State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing, 210092, China

Email: dechenjiang@nju.edu.cn

Experimental

Chemical. AChE, Acetylcholine chloride, choline oxidase and DHR123 were purchased from Sigma-Aldrich. 25% Glutaraldehyde was purchased from Sinopharm Chemical Reagent. Rhodamine B (RhB) was purchased from Sangon Biotech. Ultrapure water (Mill-Q, Millipore, 18.2 MΩ cm resistivity) was used throughout the experiments.

Preparation of the micro-capillary. A glass capillary (BF100-58-10) was pulled using a micropipette puller (P2000; Sutter Instrument) to create a tip with an opening of ~1 μm. A scanning electron microscope (SEM, Hitachi S-4800 Instrument, Japan) was used to characterize the tip of the micro-capillary.

Formation of the micro-droplets on demand. A capillary tube was filled with a physiological buffer (10 mM PBS, pH 7.4) containing all the kit components for the determination of enzymatic activity. The silver wire was placed inside the capillary tube, respectively. A voltage of 1.2 V was applied between the wire and ITO slide using an electrochemical station (CHI 630E; CH Instruments). After the collection of background current, the capillary moves down till the observation of the current increase. Under this condition, the micro-droplet is in contact with ITO surface. Then, the capillary moves up so that the micro-droplet left at the ITO surface, as reflected by the drop of the current.

Detection of AChE activity at ITO surface. A cleaned indium tin oxide electrode (ITO) was immersed in 2.5% glutaraldehyde (10 mM PBS, pH 7.4) solution for 30 minutes, and then immersed in 5 U/mL AChE (10 mM PBS, pH 7.4) in the solution for 60 minutes. The enzyme coated slide was baked in an oven at 35 °C for 30 minutes. Then, this slide was placed under the fluorescence microscopy (Olympus 51, Japan). A micro-capillary was filled with a physiological buffer (10 mM PBS, pH 7.4) containing 75 mM acetylcholine chloride, 5 U/mL choline oxidase, 20 μg/mL DHR123, and 0.1 μM RhB, which was suspended above the slide. After the formation of 25 micro-droplets at the ITO surface on demand, green and red fluorescence images were recorded using the filter sets and an EM CCD (Evolve, Photometrics, Tucson, AZ), respectively. The fluorescence ratios from these microkits in the image were analyzed using ImageJ software.

Detection of AChE activity in mouse brain slice. The frozen mouse brain slices (GemPharmatech, Nanjing, China) were used for analysis. The formation of micro-droplets at the neurons in the slice and the following fluorescence detection were the same as the protocols as mentioned above.

Figure S1. SEM characterization of capillary tip. (A) side view; (B) top view.

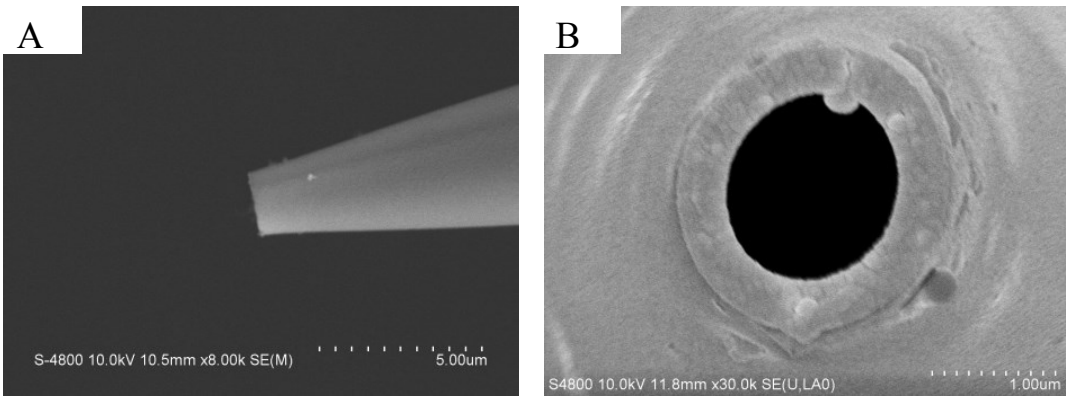

Figure S2. The bright-field, green fluorescence, red fluorescence and fluorescence ratio images from 25 microkits at the brain slice; (A) choline oxidase, DHR123 and RhB, (B) acetylcholine chloride, DHR123 and RhB, (C) DHR123 and RhB.