Supplementary Information for

Analysis of Sphingomyelin in Plasma Membrane at Single Cells using Luminol Electrochemiluminescence

Yao Chen[a],Yanan Liu[a],Juan Xia[a],Jing Liu[a],Dechen Jiang[b] and Depeng Jiang[a]*

[a] Department of Respiratory Medicine, The Second Affiliated Hospital，Chongqing Medical University, Chongqing 400010, China;

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

Email: depengjiang@163.com

Tel: 86-23-63693093; Fax: 86-23-63711527

Experiential Section.

Chemicals. Raw264.7 cells were obtained from Institute of Biochemistry and Cell Biology, Shanghai Institute for Biological Sciences of Chinese Academy of Science (Shanghai, China). Sphingomyelinase were purchased from Cayman Chemical (Ann Arbor, MI). All other chemicals were from Sigma. Ultrapure water with a resistivity of 18.2 MΩ/cm was used throughout.

Cell culture. Raw264.7 cells were seeded in DMEM/high glucose medium supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin/streptomycin) at 37°C under a humidified atmosphere containing 5% CO_2. For luminescence analysis, 20000 cells were cultured in 10 mM phosphate buffer saline (PBS, pH 7.4) on indium tin oxide (ITO) electrode at room temperature.

Luminescence detection. The ITO electrode cultured with the cells, Ag/AgCl and Pt electrodes were used as the working, reference and counter electrodes, respectively. The solution in the cell chamber was 20 μL PBS (10 mM, pH 7.4) with luminol (200 μM). All the experiments were performed in the dark room. A pipette with the mixed enzymes was placed above ITO electrode so that the enzymes could be added into the solution avoiding any ambient light. The luminescence was recorded in the potential from -1.0 to 1.0 V with a scan rate of 1 V/s. After the collecting of background luminescence, 5U/L sphingomyelinase, alkaline phosphatase and choline oxidase were introduced into the buffer for a certain time following the luminescence recording. The ratio of luminescence intensity read at 1.0 V after and before the introduction of enzyme mixture was determined as the signal. For single cell analysis, a pinhole with 100 μm in diameter was prepared using a 100 μm microdrill and placed between ITO electrode and PMT detection window. The cell density was adjusted so that the cell-cell distance was larger than 100 μm. Therefore, only one cell was positioned above the pinhole, as confirmed by the observation under the microscopy. The same detection procedure was processed and the luminescence through the pinhole was recorded reflecting the amount of sphingomyelin from the cell above the pinhole.