# **Real-time Monitoring of The Oxidative Response of A Membrane-Channel Biomimetic System to Free Radicals**

# Yu Liu<sup>a</sup>, Yi-Lun Ying<sup>a</sup>, Hai-Yan Wang<sup>a</sup>, Chan Cao<sup>a</sup>, Da-Wei Li<sup>a</sup>, Wen-Qing Zhang<sup>a</sup> and Yi-Tao Long<sup>a\*</sup>

<sup>a</sup> Shanghai Key Laboratory of Functional Materials Chemistry & Department of Chemistry, East China University of Science and Technology, 130 Meilong Road, Shanghai, 200237, P.R. China.

Email: ytlong@ecust.edu.cn

# **Supplementary Information**

## 1. Chemicals

All reagents were of analytical grade, and doubly distilled water was used throughout.  $\alpha$ -HL was purchased from Sigma-Aldrich (St. Louis, MO, USA) and was used without further purification. Diphytanoyl phosphatidylcholine was purchased from Avanti Polar Lipids Inc. (Alabaster, AL, USA). Pyrogallol was purchased from Sinopharm Chemical Reagent Co., Ltd (AP, Shanghai, China) and was dissolved in deionized water without further purification. Decane was purchased from Sigma-Aldrich ( $\geq$ 99%, St. Louis, MO, USA).

#### 2. Data analysis section

The current blockages that were larger than a threshold value of 15 pA were recorded. These events, defined by any current blockage that exceeded the threshold value, were strung together in series and each event was analyzed by the home designed software.

Reported standard deviations are based on the three separate experiments.<sup>1</sup>

### 3. The preparation of phospholipid membrane

The lipid bilayers were created by applying diphytanoyl phosphatidylcholine (30 mg mL<sup>-1</sup>) in decane ( $\geq$ 99%, Sigma-Aldrich, St. Louis, MO, USA) to a 150-µm orifice in a 1 mL bilayer chamber (Warner Instruments, Hamden, CT, USA) filled with KCl (1.0 M) and Tris-HCl (10 mM).<sup>2</sup> Unless otherwise noted, the pH of the solution is 7.98. A bilayer was deemed stably by monitoring its resistance and capacitance. The two compartments of the bilayer cell are termed *cis* and *trans* (show in Fig.1a).,

where the *trans* compartment is defined as virtual ground. The potential was applied at + 100 mV from the *cis* side by an Ag/AgCl electrode. The experiments were run under voltage-clamp conditions using a ChemClamp (Dagan Corporation, Minneapolis, MN, USA) instrument. Currents were filtered at 10 kHz by DigiData 1440A (Axon Instrument, Forest City, CA, USA) hardware and recorded by a PC running PClamp 10.2 (Axon Instrument, Forest City, CA, USA).

#### 4. The insertion of $\alpha$ -HL on phospholipid membrane

The  $\alpha$ -HL was injected adjacent to the aperture in the *cis* chamber, and pore insertion was determined by a well-defined jump in current value. Once a stable single-pore insertion was detected, the pyrogallol solution was added to the *cis* chamber. Analysis of all data was performed by ClampFit 10.2 (Axon Instruments, Forest City, CA, USA) and OriginLab 8.0 (OriginLab Corporation, North-ampton, MA, USA).

#### 5. The autoxidation of pyrogallol

The experiments were carried out at 20±0.5 °C by using a variable-temperature water-circulating bath (DKB -501A, Jinghong Co. Ltd., Shanghai, China) connected to homemade thermal stage.

After an  $\alpha$ -HL protein pore had stably inserted on phospholipid membrane, the pyrogallol was injected into the *cis* chamber with a mild stir to help it disperse homogeneously in buffer solution. The experiment was exposed to the atmosphere all the time.

In the autoxidation reaction, the oxidation substrate pyrogallol will firstly become the high oxidative activity chemicals as superoxide anion radical and semiquinone radical. This reaction will finally be ended as producing the quinone and hydrogen peroxide.<sup>3</sup>



Fig. S1 The autoxidation process of pyrogallol.

## 6. UV-Vis characterization on the autoxidation process of pyrogallol

The UV–Vis absorption spectra were collected using the USB2000+ spectrometer with an Ocean Optics DT-mini-2 halogen recourse (Ocean Optics, USA).



Fig. S2 The UV-Vis characterization of 100  $\mu$ M pyrogallol in (a) pH=5.85 Tris-HCl buffer solution and (b) pH=7.98 Tris-HCl buffer solution.<sup>4</sup>

## **Reference:**

- 1. A. Meller, L. Nivon, E. Brandin, J. Golovchenko and D. Branton, PNAS, 2000, 97, 1079-1084,
- (a) A. Meller, L. Nivon and D. Branton, *Phys. Rev. Lett.*, 2001, **86**, 3435-3438; (b) M. Akeson, D. Branton, J. J. Kasianowicz, E. Brandin and D. W. Deamer, *Biophys J.*, 1999, **77**, 3227.
- 3. L. Magnani, E. M. Gaydou and J. C. Hubaud, Anal. Chim. Acta, 2000, 411, 209-216.
- 4. X. Li, J. Agric. Food. Chem., 2012, 60, 6418-6424.