# Supplementary Information

# Pyrenecarboxaldehyde Encapsulated Porous $TiO_2$ Nanoreactors for Cellular GSH Levels Monitoring

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## 1.1 Reagents and Materials

Pyrenecarboxaldehyde (Pyc) was purchased from Adamas reagent Co., Ltd. (Shanghai, China). Potassium chloride (KCl) and urea were provided by Kelong Chemical Co., Ltd. (Chengdu, China). L-glutathione (GSH) was supplied from the J&K Chemical Ltd. (Beijing, China). L-glutamine (Gln), glycine (Gly), cysteine (Cys) and serine (Ser) were purchased from Shanghai Kangda Amino Acid Factory. Titanium isopropoxide (TIP), absolute ethanol, dichloromethane ($CH_2Cl_2$), sodium chloride (NaCl), manganese chloride ($MnCl_2$) and ascorbic acid (AA) were purchased from Titan Scientific Co., Ltd (Shanghai, China). 0.1 M phosphate buffered saline (PBS, pH 7.4) containing 10 mM $S_2O_8^{2-}$ solution was prepared for electrolyte solution. Deionized water (specific resistance of 18.2 MΩ·cm$^{-1}$) was used to prepare all the aqueous solutions.

## 1.2 Apparatus

Three-electrode system composed of an Ag/AgCl electrode (saturated KCl) (the reference electrode), a platinum wire (auxiliary electrode) and a modified glassy carbon electrode (GCE, $\Phi$ = 3 mm) (the working electrode) was used in the experiment. The ECL measurements were performed on a model MPI-E ECL analyzer (Xi'An Remax Analyse Instrument Co., Ltd., Xi'An, China). Fluorescence characterization was conducted on FL-5700 spectrophotometer (Hitachi, Tokyo, Japan). ECL emission spectra were measured on a Newton EMCCD spectroscopy detector (Andor Co., England). X-ray photoelectron spectroscopy (XPS) was measured on Thermo Scientific K-Alpha instrument (Thermo Fisher Scientific, USA).

## 1.3 Cell Lysis

Briefly, the cell pellet was added to the appropriate amount of Trizol Reagent, followed by incubation at room temperature for 5 min to ensure complete cell disruption. Then, 2-propanol was used to precipitate the RNA extraction. Finally, the extracted RNA solution was diluted with DEPC-treated water and then repackaged to 200 μL centrifuge tubes with ten microliters in each tube and stored at -20 °C for further quantification by using our proposed strategy. In the experimental operations, all appliances including the centrifuge tubes, spearheads, and culture dishes, were soaked in 0.01% DEPC solution overnight and dried in an oven after a sterilization process. The solutions used in all experiments were prepared using DEPC-treated water.

### 1.4 Statistical Analysis

The values were calculated using IBM SPSS 21 software (IBM, Armonk, NY, USA). One-way ANOVA with LSD test used for comparison, $P < 0.05$.

### 1.5 Preparation of Pyc NPs

First, 24 mg of pyrenecarboxaldehyde (Pyc) was dissolved in 500 μL of ethanol, which was then ultrasonicated for 10 min to obtain a uniform yellow solution. Then, 30 mL of a cetyltrimethylammonium bromide (CTAB, 0.18 M, cationic surfactant) solution was added into the abovementioned solution to obtain aggregated Pyc nanoparticles (Pyc NPs) in a poor solvent. The mixture was stirred at 78 °C for 20 min to encapsulate the Pyc NPs into the hydrophobic inner parts of the CTAB micelles. Finally, the prepared Pyc NPs were collected by centrifugation at 12000 rpm for 20 min and washed with deionized water several times to remove the surfactants until the pH was nearly neutral.

### 1.6 Relative ECL Efficiency of the Pyc@pTiO$_2$

The relative ECL quantum efficiency was used to evaluate the ECL performance of Pyc@pTiO$_2$. Herein, the ECL efficiency of Pyc@pTiO$_2$ relative to Pyc was calculated using the relation below:

$$Q = \frac{\Phi_x}{\Phi_0} = \left( \frac{\int_0^t I\,dt}{\int_0^t i\,dt} \right)_x \Big/ \left( \frac{\int_0^t I\,dt}{\int_0^t i\,dt} \right)_0$$

Here, $\Phi_x$ and $\Phi_0$ are the ECL quantum efficiency of Pyc@pTiO$_2$ and Pyc, respectively. Where "$I$" is ECL intensity, "$i$" is current value.

The relative ECL efficiency of Pyc@pTiO$_2$ in reference to that of Pyc was

calculated as 17.93. This result demonstrated that the hydrophilic interface and porous channels of Pyc@pTiO$_2$ could remarkably increase the ECL efficiency by concentrating reactive substances and improving the utilization ratio of reactive intermediates involved.

### 1.7 DPV Measurements of Pyc NPs and Pyc@pTiO$_2$

The differential pulse voltammetry (DPV) was carried out in tetrabutylammonium hexafluorophosphate (TBAPF$_6$) solution to investigate the ECL reaction mechanism between Pyc and TiO$_2$. As shown in **Fig. S1**, the Pyc NPs/GCE exhibited two cathodic peak at around -0.42 V and -1.03 V (**curve a**), corresponding to the emission of singlet Pyc (Pyc$^*$) and singlet oxygen ($^1(O_2)_2^*$). Compared to the DPV curve of Pyc NPs/GCE, the Pyc@pTiO$_2$/GCE (**Fig. S1**, **curve b**) exhibited two reduction peak at around -0.52 V and -1.24 V with the potential shifted by 0.10 V and 0.21V, respectively. From this result, we speculated that the high mobility of charge carrier in pTiO$_2$ might facilitate the ultimate interaction between Pyc$^{\bullet-}$ and $SO_4^{\bullet-}$ to produce excited-state Pyc$^*$ for strong ECL emission.（red line）

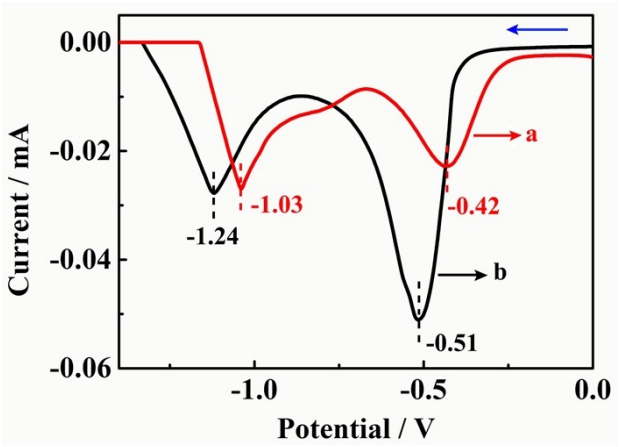


**Fig. S1** DPV curves of Pyc NPs/GCE (a) and Pyc@pTiO$_2$/GCE (b) in TBAPF$_6$ solution, the blue arrow indicated the scan direction.

### 1.8 Comparison of Other Test Platforms

**Table S1** Different biosensors with different methods for GSH detection

| Method | linear range | LOD | Ref. |
|---|---|---|---|
| Colorimetric detection | 0 μM ~ 300 μM | 65 μM | 1 |
| Lateral flow plasmonic | 25 μM ~ 500 μM | 9.80 μM | 2 |
| Biological nanopores | 0.5 mM ~ 10 mM | 0.1 mM | 3 |
| FL | 10 nM ~ 180 nM | 3. 07 nM | 4 |
| PEC | 5 μM ~ 200 μM | 2.83 μM | 5 |
| SERS | 0.1 mM ~ 15.2 mM | 0.052 mM | 6 |
| ECL | 5 μM ~ 200 μM | 0.9 μM | 7 |
| ECL | 1 μM ~ 10 mM | 0.24 μM | This work |

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