

## Supporting Information

### **Anthracene carboxyimide-based selenide as fluorescent probe for ultrasensitive detection of hypochlorous acid**

#### **Contents**

1. Preparation of reactive oxygen species (ROS) and reactive nitrogen species (RNS)
2. Determination of the detection limit
3. HR-MS data of **AC-Se** and the reaction mixture of **AC-Se** with  $\text{ClO}^-$
4. Photostability of probe **AC-Se** and the oxidation product toward  $\text{ClO}^-$
5. Cytotoxicity assays
6. Real-time imaging
7. NMR spectra

## 1. Preparation of reactive oxygen species (ROS) and reactive nitrogen species (RNS)

All the stocking solutions of ROS/RNS were prepared based on the reported literature [1]. The stock hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), sodium hypochloride ( $\text{NaOCl}$ ) and tert-Butyl hydroperoxide (TBHP) solutions were purchased from Sigma-Aldrich. Superoxide anion ( $\text{O}_2^-$ ) solution was prepared by fully dispersing the potassium dioxide in anhydrous DMSO via ultrasonic treatment. Hydroxyl radicals ( $\cdot\text{OH}$ ) and tert-butoxy radical ( $\text{tBuO}\cdot$ ) were prepared by Fenton reaction, the molar ratio of  $\text{FeSO}_4\cdot\text{H}_2\text{O}_2$  and  $\text{FeSO}_4\cdot\text{TBHP}$  was 1:10. Peroxyl radicals ( $\text{ROO}\cdot$ ) was generated from 2,2'-azobis(2-amidinopropane)dihydrochloride. Peroxynitrite ( $\text{ONOO}^-$ ) solution was prepared by 3-morpholinopyridone hydrochloride (SIN-1).  $\text{NO}\cdot$  were diluted from the commercially available 2,2'-azobis(2-amidinopropane) dihydrochloride and sodium nitroferricyanide(III) dihydrate (SNP) to ultrapure water.

## 2. Determination of the detection limit

The detection limit was calculated based on the method reported in the previous literature by the equation as follows:

$$\text{Detection limit} = 3\sigma/k [2]$$

Where  $\sigma$  is the standard deviation of blank measurement,  $k$  is the slope of the equation between fluorescence intensity and the concentrations of  $\text{NaOCl}$ . We measured the fluorescence intensity of the probe **AC-Se** without  $\text{NaOCl}$  for six times to obtain the standard deviation, and the slope  $k$  was obtained according to the linear equation of the fluorescence intensity of  $F_{542}$  with the increasing concentration of  $\text{NaOCl}$ .

### 3. HR-MS data of AC-Se and the reaction mixture of AC-Se with ClO<sup>-</sup>

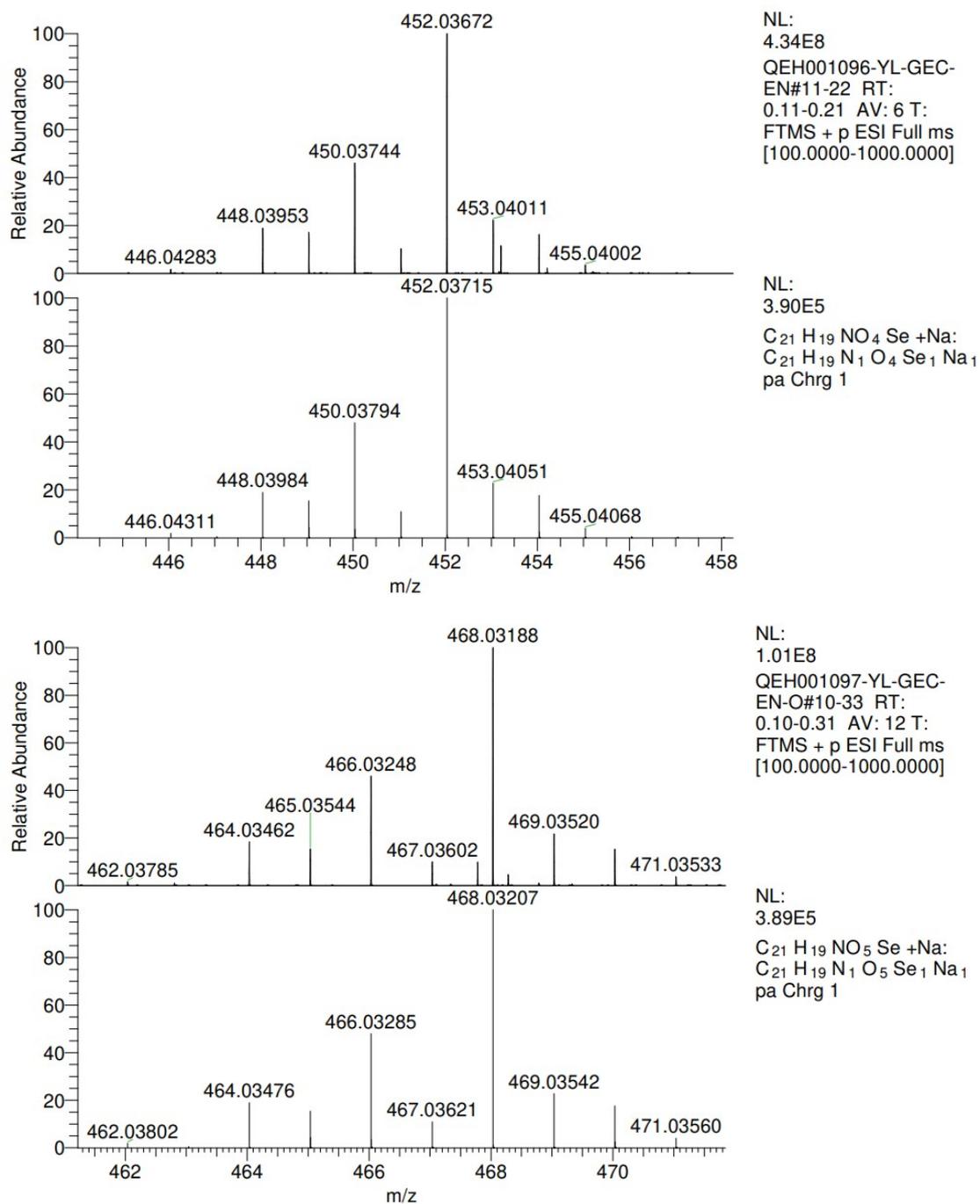
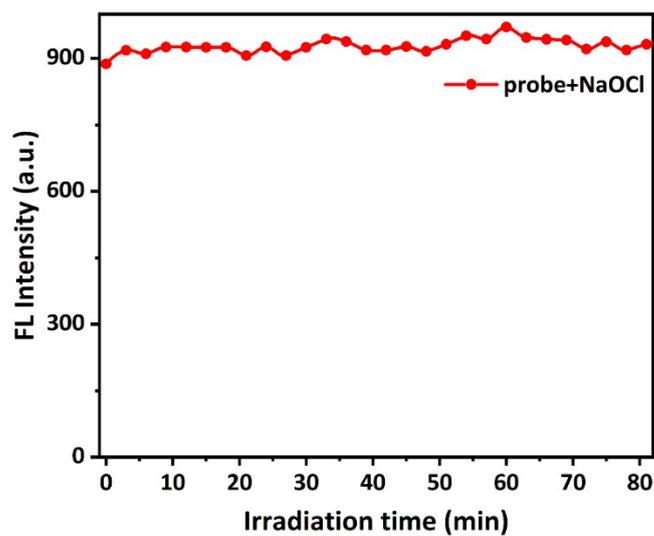


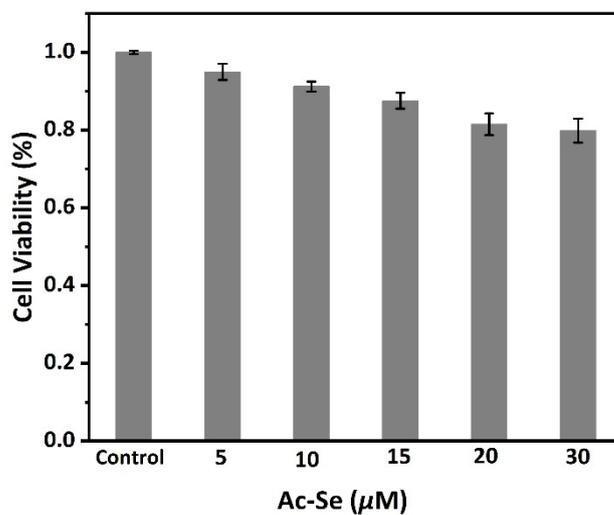
Fig. S1 HR-MS spectra of AC-Se and the reaction mixture of AC-Se with ClO<sup>-</sup>.

#### 4. Photostability of probe AC-Se and the oxidation product toward $\text{ClO}^-$



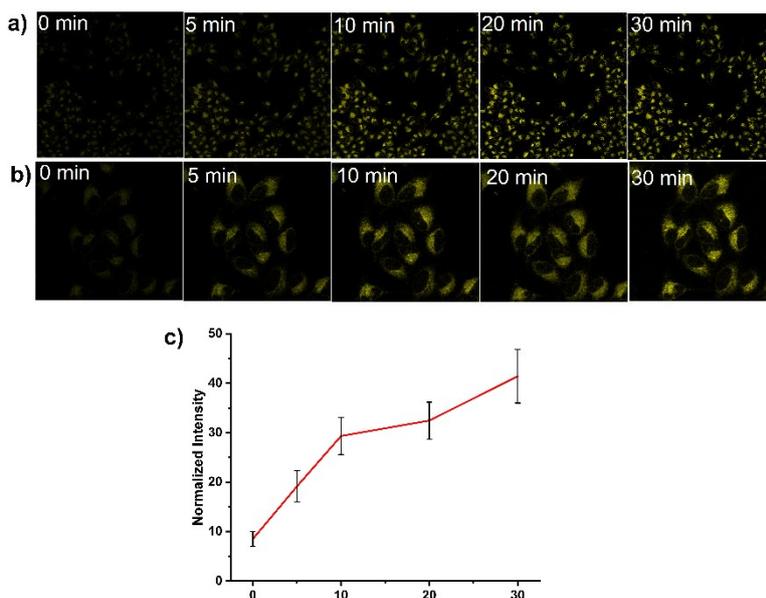
**Fig. S2** Time-dependent fluorescence intensity changes of AC-Se ( $10 \mu\text{M}$ ) under the irradiation by a 450W lamp,  $\lambda_{\text{ex}} = 470 \text{ nm}$ , slits = 2/2 nm.

#### 5. Cytotoxicity assays



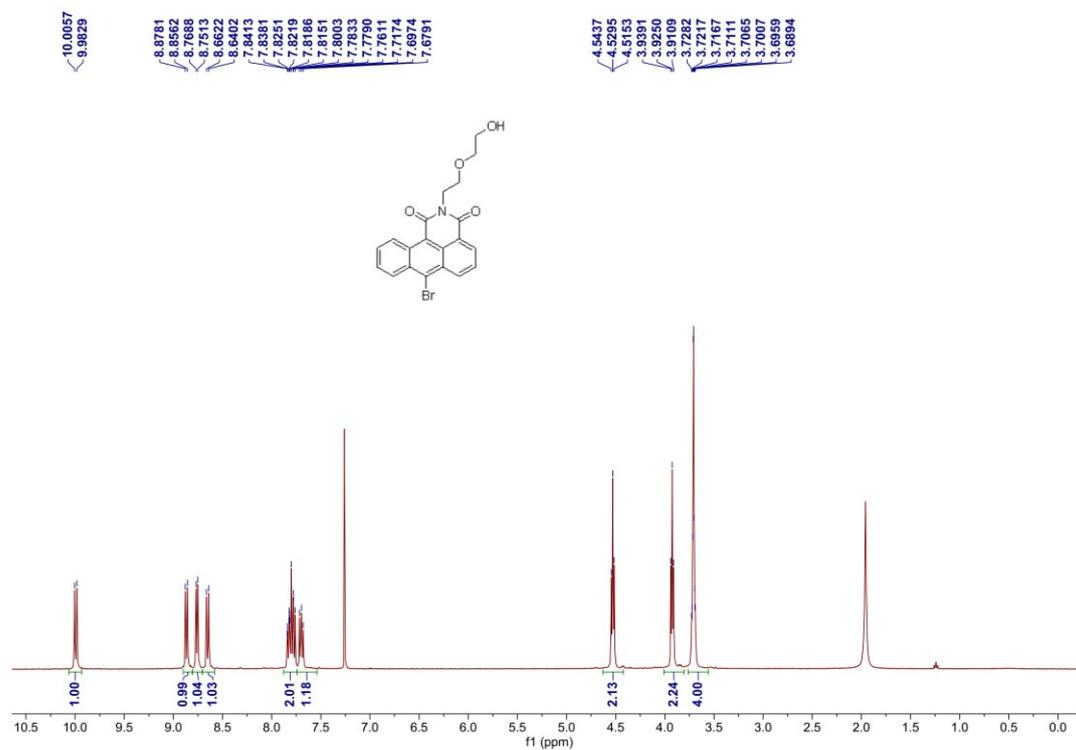
**Fig. S3** Cell viability of HeLa cells treated with different concentrations of AC-Se (0, 5, 10, 15, 20, 30  $\mu\text{M}$ ) for 24 h.

## 6. Real-time imaging



**Fig. S4** (a) Time course of fluorescence intensity (0-30 minutes) of HeLa cells after addition of AC-Se (5 μM). (b) Time course of fluorescence intensity (0-30 minutes) of local HeLa cells from Fig. S4a. (c) Average fluorescence intensity from image S4b respectively.  $\lambda_{\text{exc}} = 488 \text{ nm}$ ,  $\lambda_{\text{em}} = 540\text{-}560 \text{ nm}$ .

## 7. NMR spectra



**Fig. S5** <sup>1</sup>H NMR (400 MHz) spectrum of AC-Br in CDCl<sub>3</sub>.

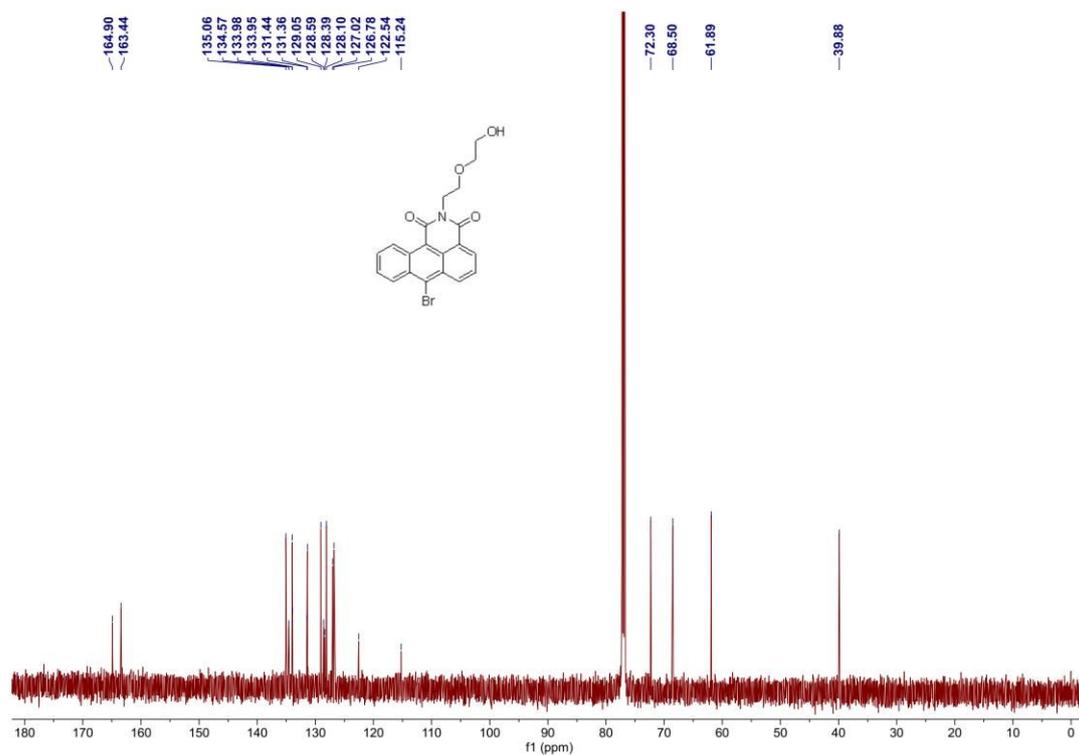


Fig. S6 <sup>13</sup>C NMR (151 MHz) spectrum of AC-Br in CDCl<sub>3</sub>.

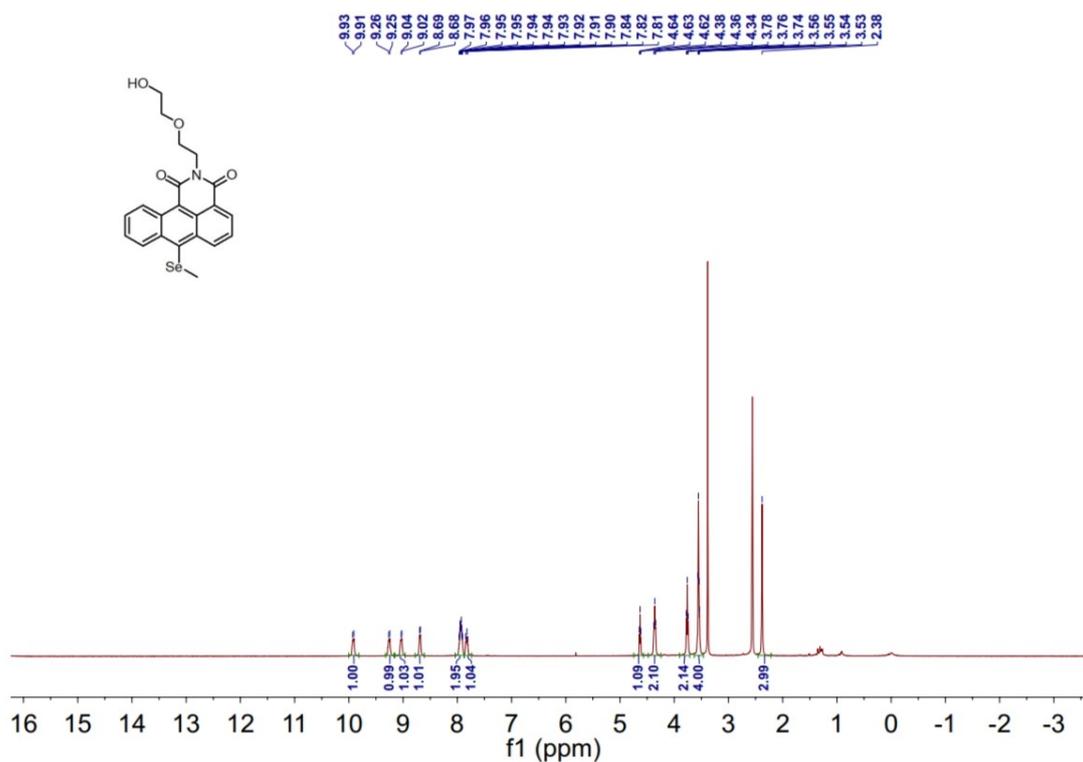
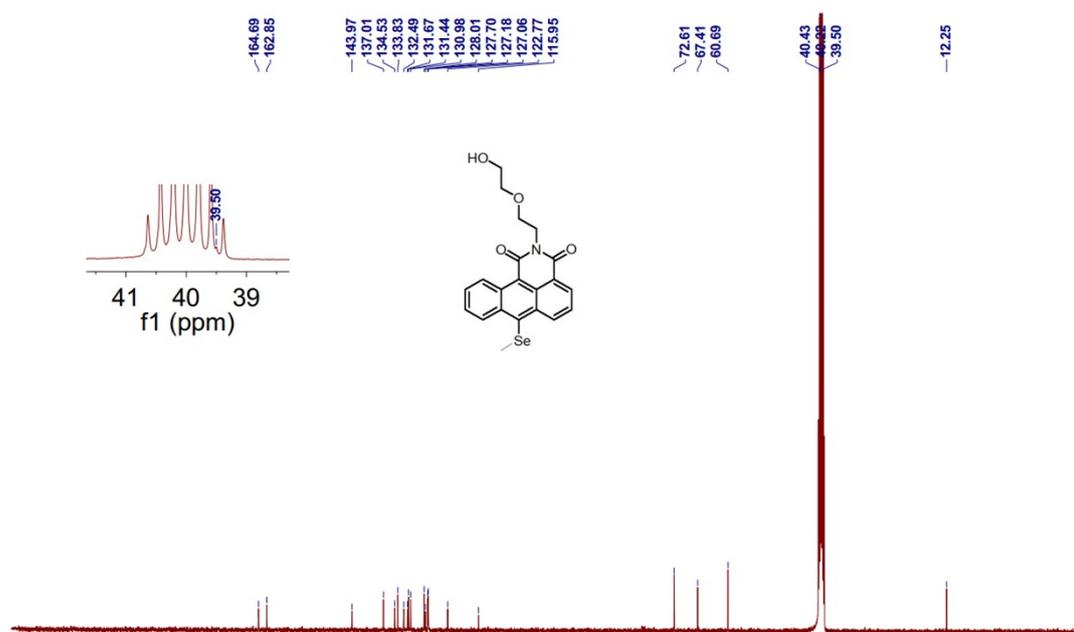


Fig. S7 <sup>1</sup>H NMR (600 MHz) spectrum of AC-Se in DMSO-*d*<sub>6</sub>.



**Fig. S8**  $^{13}\text{C}$  NMR (151 MHz) spectrum of AC-Se in  $\text{DMSO-}d_6$ .

1. Zhu, B.; Gao, C.; Zhao, Y.; Liu, C.; Li, Y.; Wei, Q.; Ma, Z.; Du, B.; Zhang, X., A 4-hydroxynaphthalimide-derived ratiometric fluorescent chemodosimeter for imaging palladium in living cells. *Chem. Commun.* **2011**, 47, (30), 8656-8658.
2. Zeng, L.; Xia, T.; Hu, W.; Chen, S.; Chi, S.; Lei, Y.; Liu, Z., Visualizing the Regulation of Hydroxyl Radical Level by Superoxide Dismutase via a Specific Molecular Probe. *Anal Chem* **2018**, 90, (2), 1317-1324.