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Electronic Supplementary Information for

Accurate detection of reactive oxygen species by tuning an elastic motif $(GPGGA)_4$ in nanopores

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Materials

The peptide GGGCEG(GPGGA)$_4$CEG (abbreviated to elastic peptide, ES peptide (>95%) was purchased from Synpeptide Co., Ltd. (China). Polyethylene terephthalate (PET) foils of 12 μm thickness and 30 mm diameter were irradiated at the linear accelerator UNILAC (GSI, Darmstadt) with swift heavy ions having an energy of 11.4 MeV per nucleon with 10^6 ions·cm^{-2} ion tracks in the central domain. Polydimethylsiloxane (PDMS) membrane was purchased from Hangzhou Baoerde Co., Ltd. (China). Hydrogen peroxide (H_2O_2), glutathione (GSH), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS) and phorbol myristate acetate (PMA) were purchased from Aladdin Co., Ltd. (China). Human microglial clone 3 (HMC3) cells were obtained from Shanghai (China). High glucose DMEM, Fetal Bovine Serum (FBS), penicillin/streptomycin, and 0.25% trypsin-EDTA were purchased from Gibco Thermo Fisher Scientific Co., Ltd. (China). ROS Kit was purchased from Nanjing Jiancheng Bioengineering Institute Co., Ltd. (China). PBS solution buffer was purchased from Solarbio Co., Ltd. (China). Calcium-AM/propidium iodide (PI) was purchased from Beyotime Biotechnology Co., Ltd. (China). All chemicals used were of chromatographic pure. Water was purified by a Milli-Q system (18.2 MΩ·cm^{-1}).

Instruments

Raman spectroscopy was conducted on a spectrometer (NanoWizard, Germany). Circular dichroism (CD) spectra were recorded on a MOS-450 CD spectrometer (Bio-Logic, France). Scanning electron microscope (SEM) images of the PET conical nanochannel were obtained with JEOL JSM-7800F (JEOL Ltd. Japan) and FlexSEM1000 II (HITACHI HIGH-TECH). X-ray photoelectron spectroscopy (XPS) was obtained with an ESCALAB250xi (Thermo Fisher Scientific, US). Surface contact angle data were obtained from KRUSS DSA 100 (KRÜSS GmbH, Germany). All current-voltage curves were measured by a Keithley 6487 picoammeter (Tektronix Inc., US). The fluorescence of intensity was measured on a microplate (Biotek, US). The fluorescence images of HMC3 cells were conducted on laser confocal microscopy (Olympus, Japan).

Methods

All experiments were performed in compliance with National Institudes of Health guide for Human Biospecimen Storage, Tracking, Sharing and Disposal. Biological and Medical Ethics Committee of Dalian University of Technology has approved the experiments, and informed consent was obtained for any experimentation with human subjects.

Raman spectroscopy experiment

The ES peptide was dissolved in H_2O (final concentration was 1 mg·mL^{-1}), and then H_2O_2 (final concentration was 10^{-9} M) was added to allow the formation of an intramolecular disulfide bond (-S-S-). Subsequently, the above solution was freeze-dried. Then the sample was placed on a glass slice and conducted on a 785-nm near-infrared diode laser, and the ES peptide in the absence of H_2O_2 was taken as a control.

CD spectroscopy experiment

The ES peptide was dissolved in H_2O (final concentration was 1 mg·mL^{-1}), and then H_2O_2 (final concentration was 10^{-6} M) was added. The above solution was transferred into a quartz cuvette (light path: 1 mm, volume: 1 mL) for CD test. CD spectra were recorded on a range of 190 to 250 nm with a 1 nm step size at room temperature. Raw data were manipulated by

smoothing and subtracting the buffer spectrum. Percentage secondary structure contents were determined by analyzing the CD data via Dichroweb.

XPS experiment

Firstly, thioglycolic acid was modified on the Au slice and then 15 mg EDC and 9 mg NHS were added to the above Au slice for 2 h at room temperature in order to activate the carboxyl groups. Subsequently, the ES peptide (0.5 $mg \cdot mL^{-1}$) was added to allow the covalent reaction between the carboxyl group and the amino residue of ES peptide. Then the ES peptide-modified Au slice was characterized by XPS at 0^o and 30^o.

Preparation and characterization of PET nanochannels

The conical nanochannel was produced according to a previous work by Apel et al. Scheme S1 shows the etching and detection apparatus, the etching process, and the following measurement process. First, both sides of the multiple ion-irradiated PET foil with 1×10^6 $ion \cdot cm^{-2}$ were treated with UV light for 30 minutes, respectively. Then the PET foil was placed between two tailor-made Teflon modules in a stainless-steel holding apparatus. Subsequently, this whole apparatus was heated to 40°C. Then the left chamber was immediately filled with the etching solution (9 M NaOH), and the other was filled with the stopping solution (1 M HCOOH + 1 M KCl). During the etching process, the potential (+1 V) was used to monitor the etching process, and transmembrane ionic current can be observed once the nanochannels open. Then, when the ionic current reached a value of approximately 2.0×10^{-5} A, the etching process was stopped by rapidly replacing the etching solution with a stopping solution. Finally, the PET membrane with conical nanochannels was washed with deionized water and stored in deionized water until application. The large opening or small opening of conical nanochannels of PET membrane was characterized by JSM-7800F. Prior to the observation for the small opening side, the sample was coated with a 1 nm thick conductive layer of Platinum by Leica EM ACE200 coater.

Preparation of ES peptide-modified PET nanochannel

After the successful fabrication of PET nanochannel, the carboxyl groups were generated on the inner wall of the nanochannel, which can be a starting group modified with ES peptide. Specifically, as shown in Scheme 1, the PET nanochannel membrane was transferred to a 2 mL water solution containing 15 mg EDC and 9 mg NHS for 2 h at room temperature in order to activate the carboxyl groups. Subsequently, the above membrane was rinsed with deionized water and added to the ES peptide solution (0.5 $mg \cdot mL^{-1}$ in water) for 24 h to allow the covalent coupling between the carboxyl group and the amino residue of ES peptide. Then the ES peptide-modified PET nanochannel membrane was washed with deionized water to remove unreacted ES peptide. The obtained ES peptide nanochannel membrane was characterized by XPS and surface water contact angle experiment.

Transmembrane ionic current measurements

The transmembrane ionic current was measured by using a picoammeter (Keithley 6487). Firstly, the ES peptide-modified PET membrane was clamped between two tailor-made Teflon modules (the diameter of center hole is 5 mm shown in Scheme S1) and served as an isolating membrane separating two Teflon modules. The chambers of two Teflon modules were filled with 0.01 M NaCl electrolyte, and then two symmetric Ag/AgCl electrodes were placed into the two chambers. A scanning voltage from -2 V to + 2 V with a step voltage of 0.2 V and a step

time of 1 s was applied across the membrane. Consequently, the application of electric voltage would lead to the movement of ions (Na^+ and Cl^-), thus generating the ionic current.

The process of sensing H_2O_2, GSH and metal ions

After the attachment of the ES peptide, the ionic current decreased, and thus can be seen as evidence of the successful modification. The ionic current obtained from the above was assigned as the initial current value. Then a series of different concentrations of H_2O_2, GSH and metal ions solutions was prepared based on a buffer of 0.01 M NaCl. To investigate the response of this PET nanochannel, the ionic currents of ES peptide-modified nanochannels upon the addition of these H_2O_2 solutions, GSH solutions and metal ions solutions were measured.

In situ detection method

To incubate living cells, the PDMS membranes (1×1 cm) were first irradiated by ultraviolet light for 2 h and immersed in the glass dishes. Then the cell suspension (HMC3, 1×10^5 cells·mL^{-1}) was added to the glass dishes to allow the cells to adhere to the surface of the PDMS membranes firmly. After incubation for 48 h, the PDMS membranes with cells were washed using sterile PBS (pH 7.4) three times carefully. Before detection, four PDMS membranes with cells were then inserted into the left chamber of the Teflon module shown in Scheme S1. Subsequently, the measurement of transmembrane ionic current was the same as mentioned above. And the PMA in 0.01 M NaCl (the final concentration of PMA is 1 μg/mL) was added to induce the production of ROS and continuously recorded for 6 h.

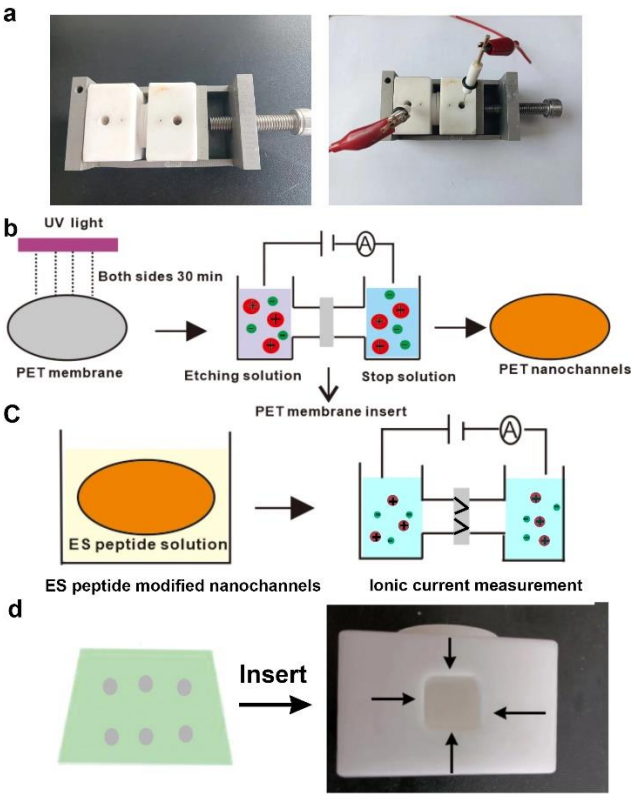
ROS detection by fluorescent probe

The ROS production released from cells, stimulated by PMA, was detected by DCFH-DA method in situ. HMC3 cells were seeded at 1×10^5 cells in a 96-well culture plate for 48 h. Then the medium was removed and the cells were washed with PBS twice. 10 μM DCFH-DA was coincubated with cells to ensure all cells are sensitive to ROS, and then PMA was added to induce ROS production. The continuous monitoring of fluorescence intensity for 6 h was performed by a multifunctional reader.

Confocal laser scanning microscopy

Confocal laser scanning microscopy images were recorded on an Olympus confocal laser scanning microscope. A 488 nm laser was chosen as the excitation wavelength of Calcium-AM and the fluorescence emission was collected at 515–540 nm. A 543 nm laser was chosen as the excitation wavelength of PI and the fluorescence emission was collected at 600–650 nm.

Figures and tables



Scheme S1 a, The schematic diagram of the etching and measurement device. b, The etching process of multiple conical nanochannels. c, The preparation of ES peptide-modified PET nanochannels. d, The strategy for living cells inserted into the modules.

Table S1. XPS peak table of bare PET.

Name	Peak BE	FWHM eV	Area (P) CPS. ev	Atomic %
S2p	167.54	0.13	360.91	0.08
C1s	284.79	1.59	166395.1	71.62
N1s	399.78	1.31	3312.5	0.87
O1s	531.88	1.62	175081.41	27.43

Table S2. XPS peak table of GGGCEG(GPGGA)$_4$CEG PET.

Name	Peak BE	FWHM eV	Area (P) CPS. ev	Atomic %
S2p	168.88	0.16	387.11	0.09
C1s	284.83	1.58	157216.71	72.73
N1s	399.87	1.46	4573.04	1.29
O1s	531.98	1.53	153760.25	25.89

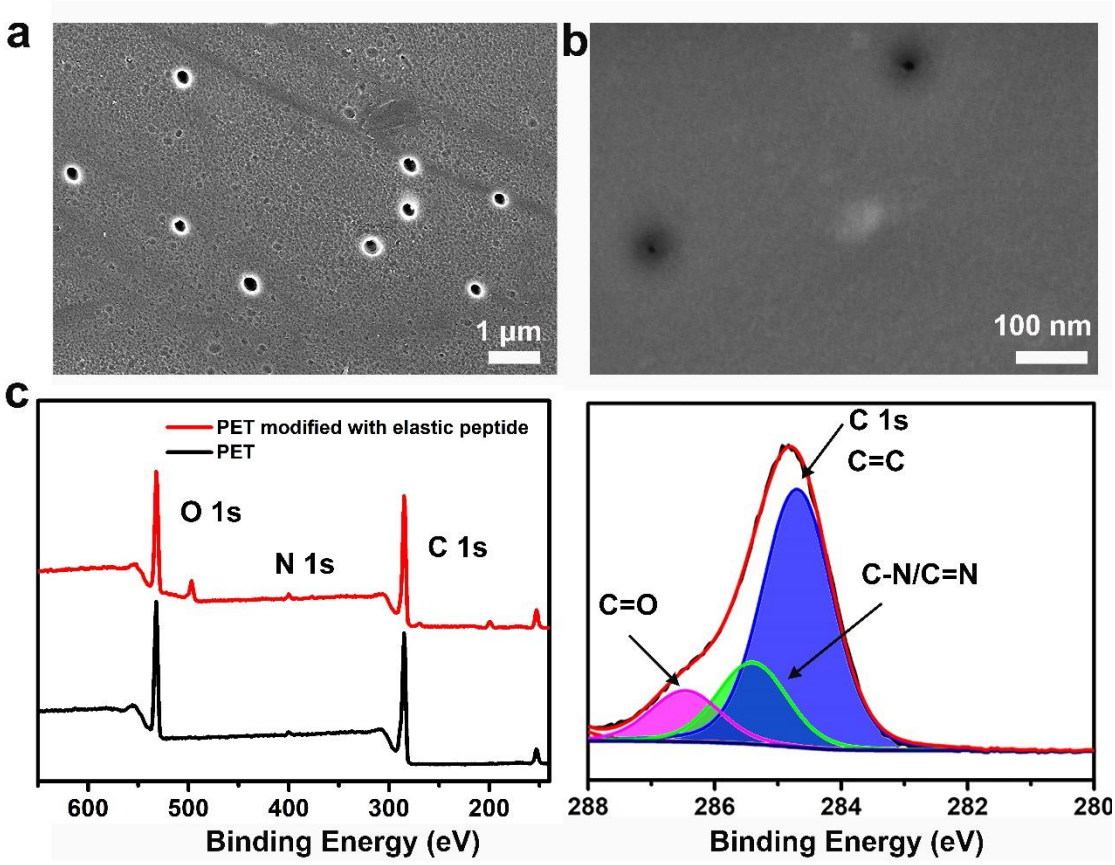


Fig. S1 Characterization of PET membrane. a, b, TEM of pore size of the large opening (a) or small opening (b) of PET membrane nanochannel. c, XPS measurement of PET membrane before (blank line) or after (red line) modified with ES peptide.

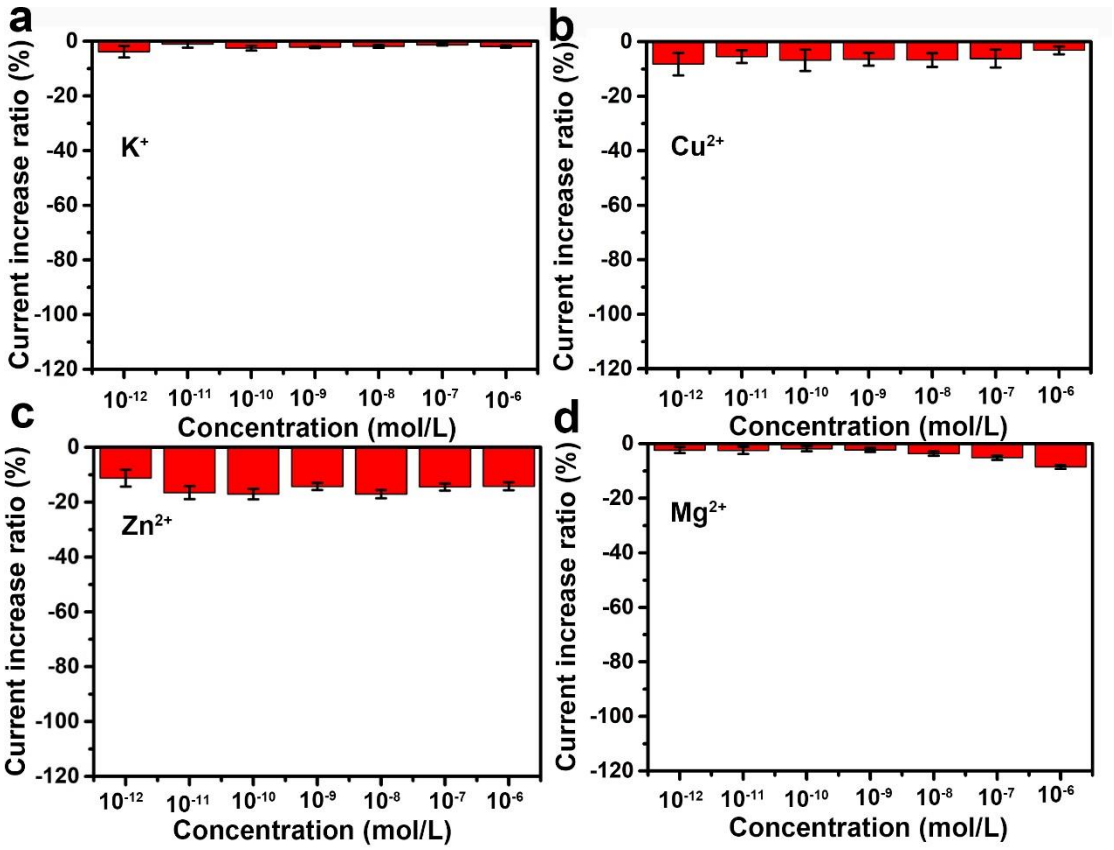


Fig. S2 Concentration-dependent ionic current increase ratio (at -2 V) of the ES peptide-modified PET nanochannel membrane in 0.01 M NaCl solution upon a series of K^+ (a), Cu^{2+} (b), Zn^{2+} (c) and Mg^{2+} (d).

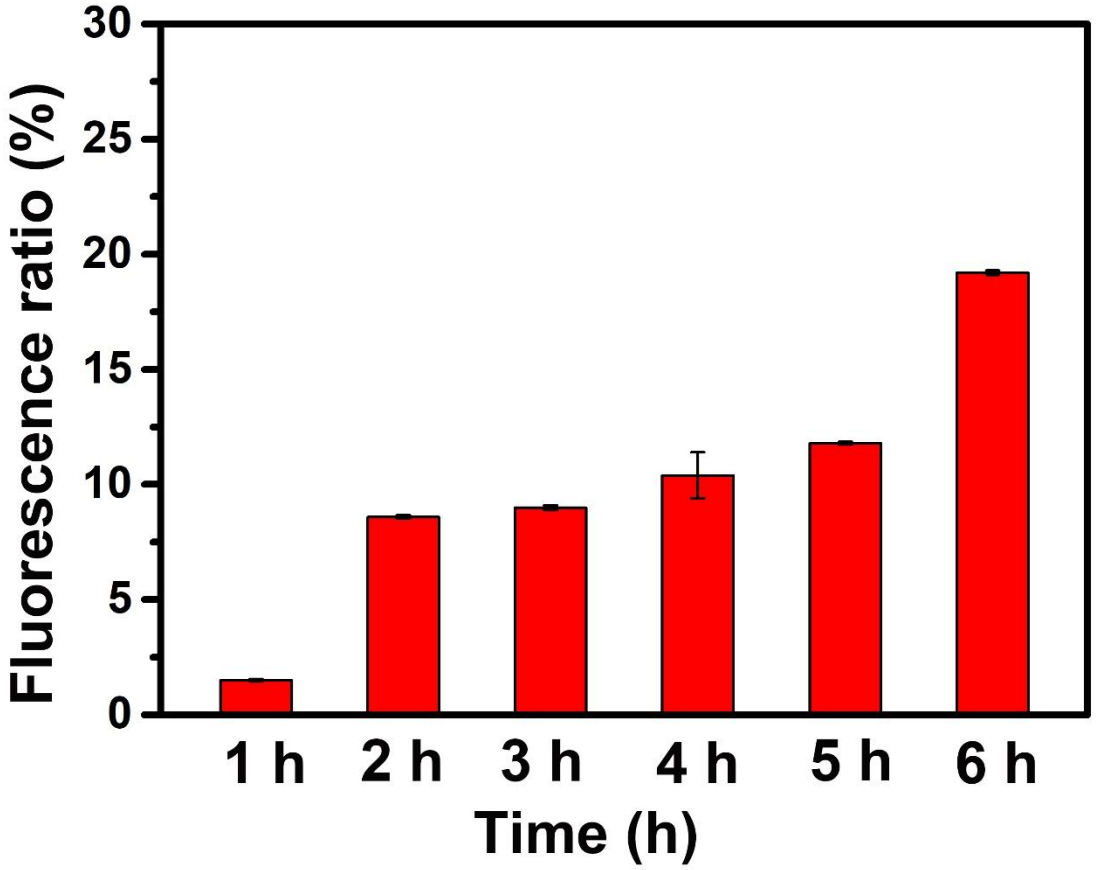


Fig. S3 The change in fluorescence intensity of cells when treated with PMA for 6 h.