# **Supplementary Information**

# Degradation of phthalic acid esters (PAEs) by enzyme mimics and its

# application to degradation of intracellular DEHP

# Experiment

### Materials.

Dibutyl phthalate (DBP), Dimethyl phthalate (DMP) and Di(2-ethylhexyl) phthalate (DEHP) were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China) with purity of 99%. DEHP standard, Phthalic acid (PA) standard and tyrosinase from mushroomall were purchased from Sigma-Aldrich (St. Louis, USA). The peptides used in this work were obtained from Sangon Biotech. Co., Ltd. (Shanghai, China). HeLa cells were purchased from Cell Bank of Chinese Academy of Science. All the cell culture reagents, unless otherwise stated, were purchased from Gibco (USA).

### Characterization.

Transmission electron microscopy (TEM) images were obtaind with a JEOL JEM-2100 electron microscope (Japan). Circular dichroism (CD) spectra were acquired with a JASCO J-715 spectropolarimeter (Japan). X-ray diffraction (XRD) measurement were tested on a D8 ADVANCE diffractometer (Bruker Co., Germany) using Cu K $\alpha$  (0.15406 nm) radiation. Dynamic light scattering (DLS) and Zeta potential were measured using a Zetasizer Nano ZS (Malvern Instruments Ltd., UK).

# **Preparation of enzyme mimics**

Purified lyophilized peptides were first dissolved in the buffer (10 mM) to obtain peptides solution (0.5 mM). To obtain self-assembly peptides, the above solutions were stored at room temperature for 24 h. To obtain chemoenzymatic polymerized peptides, tyrosinase (10 KU/mL) was added to the above solutions, followed by stirred at room temperature. After 12 h, the resulting solutions were centrifuged (12000 rpm for 10 min), and the precipitates were washed with cold Milli-Q (4 °C) to remove the tyrosinase. To obtain FITC labed enzyme mimics, the prepared enzyme mimics was dissolved in the phosphate buffer solution (50 mM, pH 9) containing FITC (1.0 mM), and then the resulting solutions were stirred at 4 °C for 6 h and dialyzed to remove the unconjugated FITC.

#### **Degradation of PAEs in solution**

The degradation assay was performed in a glass vial containing enzyme mimics (1.0 mg/mL) and PAEs (200 mg/L). Non-added enzyme mimics was used as a control. All the experiments were performed in triplicate. After degradated a designated time, the residual PAEs were collected by n-hexane and measured on Gas chromatography and mass spectrometry (GCMS-QP2010 Ultral, Shimadzu, Japan) with a DB-5 column (0.25  $\mu$ m × 0.25 mm × 30 m). For the detection conditions, helium acted as a carrier gas at flow rates of 1.0 mL/min, the injection temperature was set at 250 °C and the oven temperature program was arranged from 100 °C (for 3 min) to 300 °C (for 7 min) at a heating rate of 10 °C/min. Mass spectra were collected using the electron ionization (EI) mode of 70 eV electron impact ionization. The scans mode within a range of 25-550 m/z was applied to

identify the metabolites by comparing the results with both the standard solution and the mass spectra library in the MS system. Degradation rates was acquired by measuring the elimination of PAEs in the solution and can be calculated as follows:

$$Degradation rates(\%) = \frac{C_t}{C_0} \times 100$$

where  $C_0$  = Concentration of PAEs in the control group (mg/L).

Ct = Concentration of PAEs in the test solution.

### **Molecular simulation**

Model of SL (Ac-SHDLKLKLKL-CONH<sub>2</sub>) was generated by the Chimera 1.12 program.<sup>1</sup> Its stable conformation was obtained by molecular dynamics using Gromacs 2018.3 package.<sup>2</sup> The Gromos96 54a7 force field was applied to a cubical periodic box with a 1.0 nm solute-wall distance, which contained SL filled with SPC/E water and counterions. The energy of the box was optimized using the steepest descent method, with a maximum step of 5000, up to a maximum force  $F_{\text{max}}$  of no more than 1000.0 kJ mol<sup>-1</sup> nm<sup>-1</sup>. Then, the box was equilibrated under the canonical and isothermal-isobaric ensembles. After each equilibration for 100 ps, production runs of 1 µs were performed.

Models of PAEs, including DEHP (CID: 8343), DBP (CID: 3026), DMP (CID: 8554), were downloaded from the PubChem database (<u>https://pubchem.ncbi.nlm.nih.gov/</u>). The AutoDockTools 1.5.6rc3 software was used to produce the PDBQT format files of all PAEs and SL.<sup>3</sup> Then, their interactions were studied using the AutoDock Vina program.<sup>4</sup> The docking box centred on histidine in peptide was used with a size of  $10 \times 10 \times 10$  Å<sup>3</sup>. The exhaustiveness was set to 10. The number of alternative conformations was set to 20, and the results were sorted by docking energy. Finally, the molecular docking results were visualizd by Chimera 1.12 and LigPlus 2.1 packages.<sup>1, 5</sup>

#### Cell viability assay

HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum at 37 °C. For cell viability assay, the cells were seeded in 96-well plates at density of  $5.0 \times 10^3$  cells per well. After 12 h incubation, the cells were exposed to enzyme mimics and DEHP with various concentrations and cultured for another 24 h and 48 h. Thereafter, 5 mg/mL MTT solutions was used to treat the cells for another 4 h. After that, dimethyl sulfoxide (DMSO) was added to dissove the formazan crystals. The absorbance of supernatants was read at 490 nm by a microplate reader (BIO-RAD, Japan).

# **Degradation of intracellular DEHP**

HeLa cells were seeded in six-well plates at density of  $2.0 \times 10^5$  cells per well for 12 h and further incubated with DEHP (10 mg/L). Then the enzyme mimics (100 µg/mL) was added to the plates for degradation of DEHP. Non-added enzyme mimics was used as a control. After 24 h and 48 h, the cells were trypsinized and collected, washed with PBS, ultrasonic decomposed, and then used freeze-thawing methodes to get cell lysates. The residual DEHP were collected by n-hexane and analyzed using GC/MS. For cellular uptake test, cells were treated with FITC labed enzyme mimics (5 µg/mL) for 4 h, followed by washed with PBS and imaged using fluorescence microscope (Nikon, Japan).



Scheme S1 The enzyme mimics of PGSL (a) and formation of oxyanion hole (b) by NH groups from Gly and Ser.



Fig. S1 Degradation of DEHP by LKLKLKL and tyrosinase.



Fig. S2 GC-MS chromatograms of DEHP and its degradation intermediates. (a) Control, (b) DEHP degradation intermediates after 24 h degradation by PGSL.



Fig. S3 Degradation rates of DEHP, DBP and DMP by SL



Fig. S4 Root Mean Square Deviation (RMSD) of SL in MD simulation.



Fig. S5 The 2D diagrams of PAEs-enzyme mimics interactions. Hydrogen bonds between ligand and receptor are shown in green dotted lines, while spoked arcs represents the amino acid residues producing hydrophobic interactions.



Fig. S6 (a) DLS and (b) Zeta potential of the PGSL formed in Tris buffer at pH 9.



Fig. S7 TEM images of enzyme mimics. (a), (b) and (c) were the enzyme mimics obtained in Tris buffer at pH 7, 8, and 9 respectively; (d), (e) and (f) were the enzyme mimics obtained in phosphate buffer at pH 7, 8, and 9 respectively. Scale bar: 200 nm.



Fig. S8 XRD patterns of enzyme mimics formed in (a) Tris buffer (T) and (b) phosphate buffer (P) at pH 7, 8, and 9 respectively. CD spectroscopies of enzyme mimics formed in (c) Tris buffer (T) and (d) phosphate buffer (P) at pH 7, 8, and 9, respectively.



Fig. S9 Cell viability assay of different concentrations of DEHP.

# References

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