Supplementary Information

Efficient and stable radiolabeling of polycyclic aromatic hydrocarbon assemblies: *In vivo* imaging of diesel exhaust particulates in mice

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1. General method

1.1. Materials and methods

Diesel particulate matter (NIST®, SRM®2975), chloramine-T, 1-pyrenemethylamine hydrochloride, 4-iodobenzoic acid and sodium metabisulfite were purchased from Sigma-Aldrich Korea. The detailed information about diesel particulate matter (SRM®2975) is located at the following website address (https://www-s.nist.gov/srmors/certificates/2975.pdf). *N*-succinimidyl-4-(tributylstannyl)benzoate was purchase from Synthonix. [^{125}I]NaI (in aqueous NaOH) was supplied by New Korea Industrial Co., Ltd. All commercially available reagents were used without further purification steps. The amount of radioactivity was measured by γ-counter (1480 Wizard 3, PerkinElmer, USA). ^{1}H NMR and ^{13}C NMR spectra were measured on a JEOL 500 MHz spectrometer. High-resolution mass spectrum data was obtained by using JMS-700 (JEOL, Japan). HPLC purification and analysis of the products were carried out using Agilent HPLC system. 0.1% formic acid containing H_2O (solvent A), 0.1% formic acid containing acetonitrile (solvent B) were used as eluents for all HPLC experiments. The flow rate was 1 mL/min for analytical HPLC (Eclipse XDB-C18, 4.6 x 250 mm, 5 μm) and 10 mL/min for preparative HPLC (Eclipse XDB-C18, 21.2 x 150 mm, 7 μm). SEM images were observed using a FEI Verios 460L field emission scanning electron microscope (SEM) under high performance conditions with accelerating voltages of up to 15 kV. Intratracheal instillation of the radiolabeled product was carried out by using a visual instillobot (Sejong Bio, Republic of Korea). Single photon emission computed tomography (SPECT) images were obtained by using a small animal SPECT/CT system (Siemens, USA)

1.2. Animal study

ICR male mice (six-weeks old) were purchased from Orientbio Co., Ltd. All animal protocols in the experiments were approved by the Institutional Animal Ethical Committee and performed in strict compliance with the guidelines prescribed by the committee.

2. Synthesis of the compounds

2.1. Synthesis of the stannylated precursor (2)

To a solution of 1-pyrenemethylamine hydrochloride (141 mg, 0.5 mmol) in dimethylformamide (DMF, 3 mL), *N*-succinimidyl-4-(tributylstannyl)benzoate (255 mg, 0.5 mmol) and *N,N*-diisopropylethylamine (215 µL, 1.25 mmol) were added at room temperature (Figure S1). The reaction mixture was stirred for 2 h at room temperature and then it was then diluted with ethyl acetate and brine. The resulting layers were separated and the aqueous phase was extracted with ethyl acetate. The combined organic phase was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was recrystallized by using ethyl acetate/hexanes to give **2** as white solid (233 mg, 67%). ^{1}H-NMR (500 MHz, $CDCl_3$): 8.00-8.34 (m, 9H), 7.69 (d, 2H, J = 7.5), 7.48 (m, 2H), 6.46 (t, 1H, J = 5.5), 5.35 (d, 2H, J = 5.5), 1.42-1.58 (m, 6H), 1.25-1.32 (m, 6H), 0.97-1.10 (m, 6H), 0.85 (t, 9H, J = 7.8); ^{13}C-NMR (125 MHz, $CDCl_3$): 136.72, 133.78, 131.43, 131.34, 131.08, 130.85, 129.26, 128.47, 127.71, 127.43, 126.22, 126.07, 125.53, 125.50, 125.18, 124.90, 122.93, 42.71, 29.09, 27.39, 13.74, 9.71; HRMS ($[M+H]^+$) calculated for $C_{36}H_{44}NOSn^+$: 626.2440; found: 626.2448.

2.2. Synthesis of the standard compound (1)

To a solution of 1-pyrenemethylamine hydrochloride (67 mg, 0.25 mmol) in dry DMF (3 mL), 4-iodobenzoic acid (62 mg, 0.25 mmol), HBTU (104 mg, 0.275 mmol) and DIPEA (128.5 µL, 0.75 mmol) were added at room temperature. The reaction mixture was stirred for 2 h at room temperature. After the reaction was completed, aqueous acetic acid solution (1.0 M, 50 µL) was added to the reaction solution. The crude product was purified with a preparative HPLC (eluent gradient: 40% solvent B in solvent A for 0-2 min; 100% solvent B in solvent A for 2-25 min; 100% solvent B in solvent A for 25-35 min; retention time: 23.0 min). The solvent was removed under reduced pressure to give the product **1** as white solid (69 mg, 56%). ^{1}H-NMR (500 MHz, DMSO-d_6): 9.37 (t, 1H, J = 5.8), 8.56 (d, 1H, J = 9.5), 8.37-8.31 (m, 4H), 8.22 (s, 2H), 8.13 (t, 2H, J = 7.8), 7.93 (d, 2H, J = 8.5), 7.78 (d, 2H, J = 8.5), 5.28 (d, 2H, J = 5.0); ^{13}C-NMR (125 MHz, DMSO-d_6): 166.14, 137.76, 134.22, 133.30, 131.32, 130.83, 130.64,

129.87, 128.63, 128.13, 127.92, 127.58, 127.21, 126.79, 125.79, 125.71, 125.25, 124.57, 124.47, 123.74, 99.48, 41.63; HRMS ([M]$^+$) calculated for $C_{24}H_{16}INO$: 461.0277; found: 461.0281.

3. Radiochemistry

3.1. Synthesis of ^{125}I-labeled pyrene ([^{125}I]1)

To a solution of the precursor **2** (1 mg) in 100 µL of DMSO, acetic acid (3 µL), [^{125}I]NaI in aqueous NaOH (15 µL, 55.5 MBq) was added at room temperature (Figure S1). Then, chloramine T solution (1 mg/10 µL water) was added to the reaction mixture. The labeling reaction was carried out for 30 min at room temperature and then it was quenched by addition of sodium metabisulfite solution (2 mg/10 µL water). The crude product was purified by using preparative HPLC (flow rate: 10 mL/min, eluent gradient: 40% solvent B in solvent A for 0-2 min; 100% solvent B in solvent A for 2-25 min; 100% solvent B in solvent A for 25-35 min; retention time: 23.2 min) to give ^{125}I-labeled pyrene ([^{125}I]**1**) was obtained with 32% of radiochemical yield (17.8 MBq). Radiochemical purity was >99% as determined by analytical radio-HPLC (flow rate: 1 mL/min, eluent gradient: 40% solvent B in solvent A for 0-2 min; 100% solvent B in solvent A for 2-25 min; 100% solvent B in solvent A for 25-35 min; retention time: 15.4 min).

For solid-phase extraction, the collected [^{125}I]**1** solution was diluted with water (30 mL) and then it was added to tC18 light cartridge (preconditioned with ethanol and water). The cartridge was washed with deionized water (30 mL) and then [^{125}I]**1** was eluted by adding absolute ethanol (3 mL). The solvent was evaporated under vacuum.

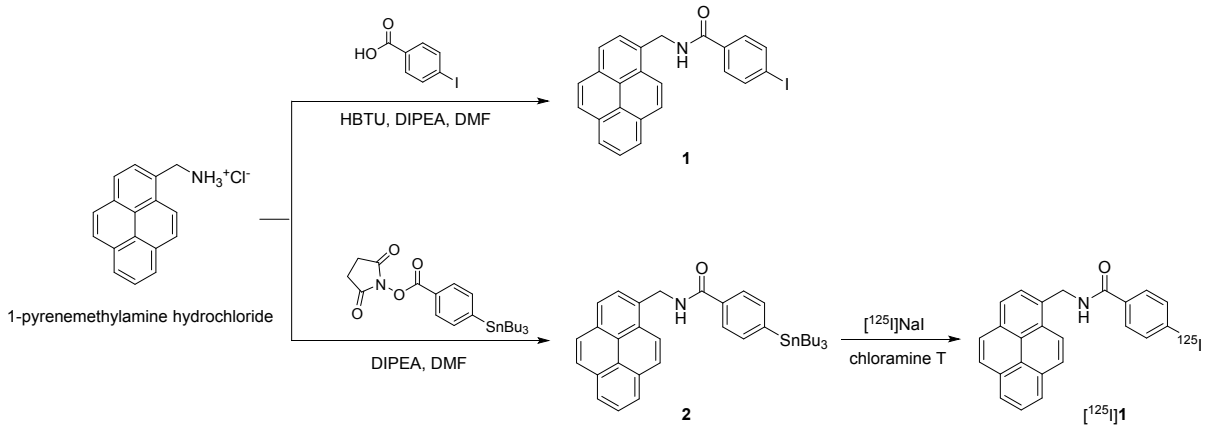

Figure S1. Scheme for synthesis of the compound **1** and [^{125}I]**1**

3.2 Preparation of ^{125}I-labeled diesel exhaust particulates (DEP) (^{125}I-DEP)

NIST® SRM®2975 powder (1.5 mg) was dissolved in DCM (4 mL) by sonication for 1h at room temperature. To this solution, [^{125}I]**1** solution (17.0 MBq, 500 μL in DCM) was added at room temperature. The solvent in the reaction mixture was removed by blowing nitrogen gas for 20 min. The dried mixture was resuspended in distilled water (3 mL) by sonication for 2 h at 75 °C. ^{125}I-DEP suspension was purified by centrifugation (13,000 rpm, 30 min, 4 °C) The pellet was collected and resuspended in distilled water for animal experiment.

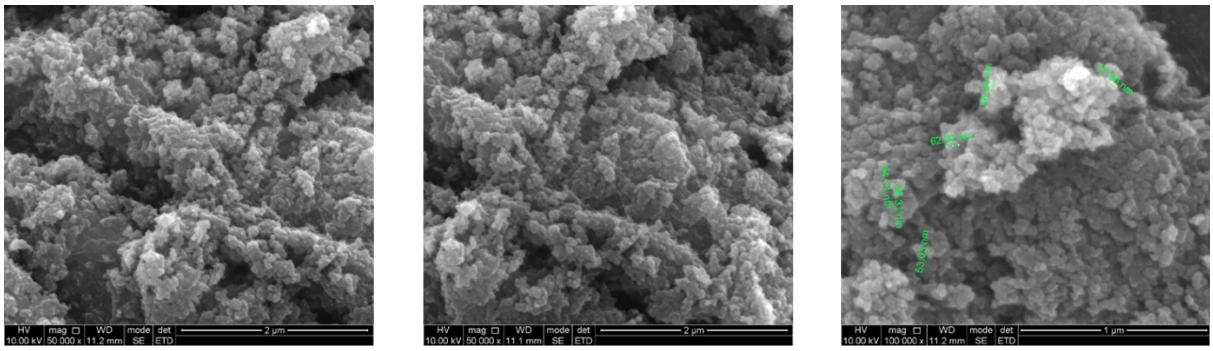

Figure S2. SEM images of DEP suspension in water

4. *In vitro* stability of ^{125}I-DEP.

^{125}I-DEP suspension (3.7 MBq/100 μL) was incubated in normal mouse serum (1 mL) at 37 ºC, 168 h. An aliquot (100 μL) of ^{125}I-DEP in serum was taken at given different time points (1.5, 5, 21, 28, 48, 72, 96, 168 h) and it diluted with water (400 μL). The resulting mixture was centrifuged with molecule weight cut off 5 K filter. The radioactivity in the pellet and supernatant were measured by γ-counter. The stability of ^{125}I-DEP in mouse serum was calculated by the following equation (1). The results were shown in figure S3.

$$\text{Stability (\%)} = C_p / (C_p + C_s) \times 100 \qquad (1)$$

(C_p = radioactivity in the pellet, C_s = radioactivity in the supernatant)

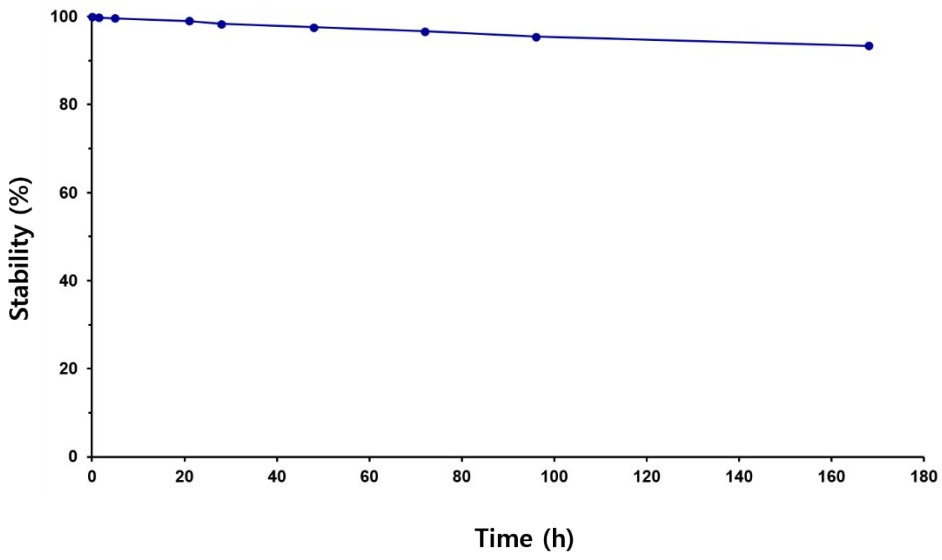

Figure S3. *In vitro* stability of ^{125}I-DEP in serum

5. Intratracheal instillation of ^{125}I-DEP in mice

For biodistribution study, ^{125}I-DEP suspension (42.5 KBq/33 μg/50 μL) was intratracheally instilled by using a visual instillobot under isoflurane inhalation anesthesia. At each time point (0.5, 2, 4, 18, 48 h), four mice were sacrificed and the organs (heart, lungs, liver, spleen, kidneys, stomach, small intestine, large intestine, and thyroid) and the blood were then harvested. The radioactivity of the harvested organs was measured by γ-counter. The distribution data were shown as a percent of the injected dose (Figure 3b) and as a percent of injected dose per gram tissue (Figure S4).

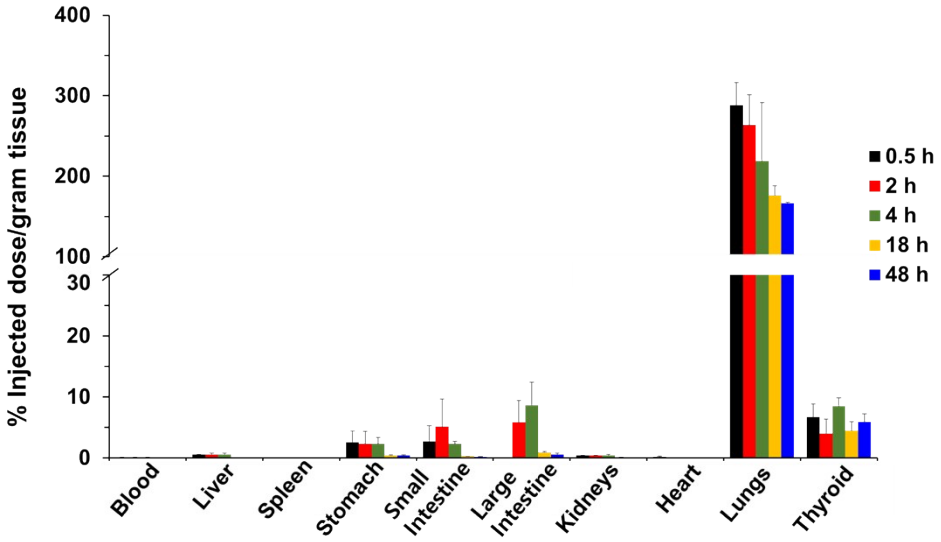

Figure S4. Biological distribution of intratracheally instilled ^{125}I-DEP in nine different organs and the blood (n = 4). Data are expressed as a percent of the injected dose per gram tissue.

6. Single photon emission computed tomography (SPECT/CT) imaging.

For imaging study, ^{125}I-DEP suspension (1.5 MBq/33 μg/50 μL) was intratracheally instilled by using a visual instillobot under isoflurane inhalation anesthesia (figure 3a) or was administrated by oral gavage (figure 3c). SPECT/CT images were acquired at 2, 18 and 48 h post administration.

7. Biodistribution of intratracheally instilled [^{125}I]**1**.

[^{125}I]**1** (37.5 KBq/33 μg/50 μL), formulated by 10% ethanol in saline, was intratracheally instilled by using a visual instillobot under isoflurane inhalation anesthesia. At each time point (0.5, 2, 4, 18, 48 h), four mice were sacrificed and the organs (heart, lungs, liver, spleen, kidneys, stomach, small intestine, large intestine, and thyroid) and the blood were then harvested. The radioactivity of the harvested organs was measured by γ-counter. The distribution data were shown as a percent of the injected dose (Figure S5).

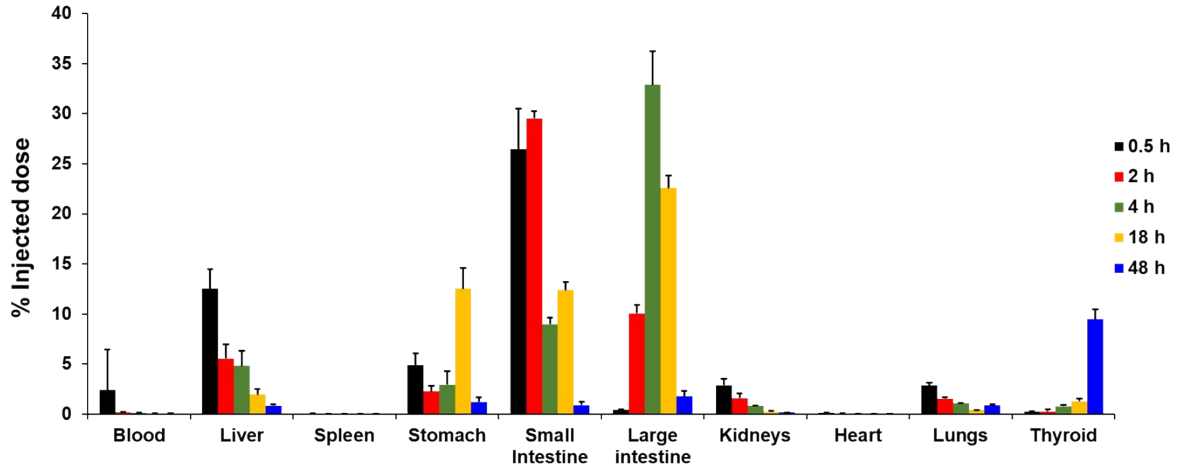

Figure S5. Biodistribution of intratracheally instilled [^{125}I]**1**

8. Cytotoxicity test of the iodine-labeled pyrene (1)

Murine bone marrow-derived macrophages and dendritic cells were separated and cultured according to the method reported previously.[S1, S2] Human hepatoma cells (HepG2) were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Carsbad, CA, USA) supplemented with 10% fetal bovine serum (Gibco BRL, 100 units/mL of penicillin/streptomycin (Gibco BRL) at 37 ^{o}C under 5% CO_2. Cell viability was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, the cells were seeded in a 48-well plate (Macrophage; 2 x 10^5 cells/mL, dendritic cells; 1 x 10^6 cells/mL, HepG2; 2 x 10^5 cells/mL) and incubated with varied concentration (0.05 – 10 μM)

of the iodine-labeled pyrene (**1**) for 24 h. After incubation, the medium in the plate was removed, and the cells were treated with MTT solution (0.5 mg/mL) for 2 h. The insoluble formazan crystal was dissolved in dimethyl sulfoxide, and then, the absorbance was measured at 570 nm using SpectraMax M3 multi-detect microplate reader (Molecular Devices, Sunnyvale, CA, USA).

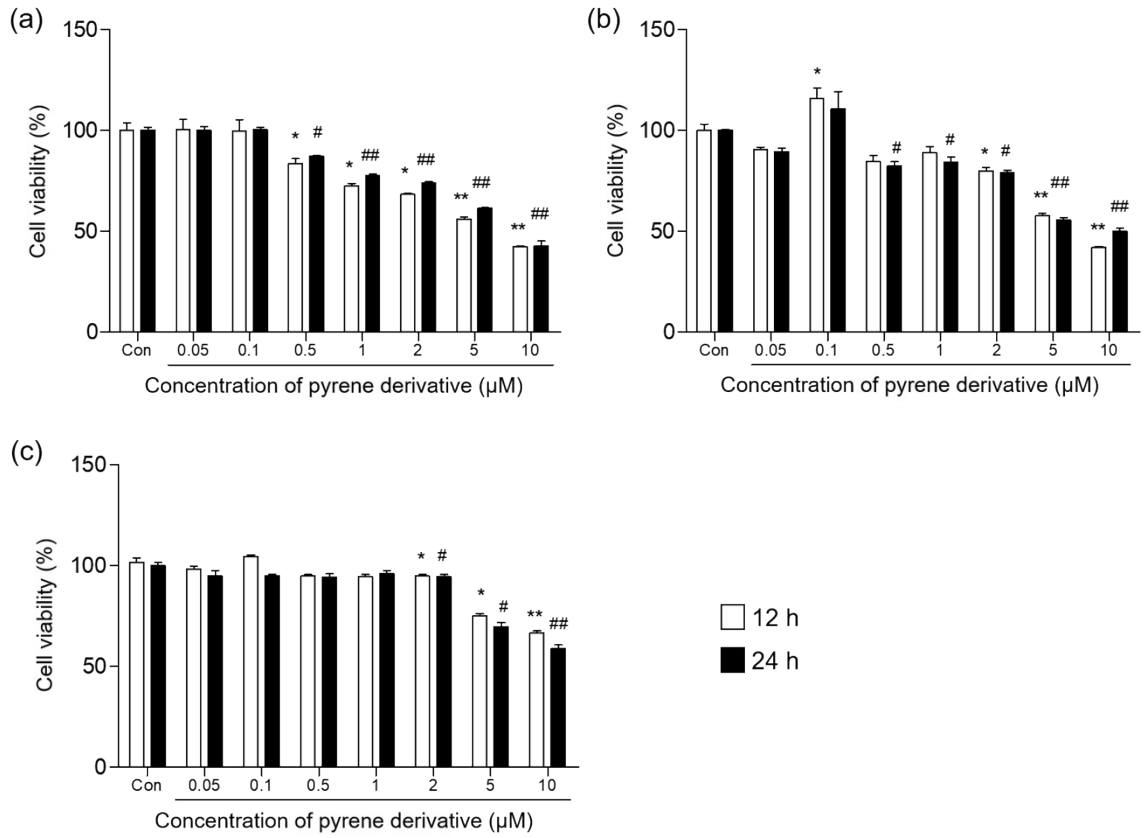

Figure S6. Cytotoxicity test of the iodine-labeled pyrene (**1**) in (a) macrophage cells, (b) dendritic cells and (c) HepG2 cells at 12 h and 24 h post incubation. The data represent mean ± SD (n = 3). Statistical analysis was performed by one way ANOVA followed by Tukey's multiple comparison test; $^{*}p < 0.05$, and $^{**}p < 0.01$ compared to control group in 12 h. $^{\#}p < 0.05$, and $^{\#\#}p < 0.01$ compared to control group in 24 h.

9. References

[S1] X. Zhang, R. Goncalves, and D. M. Mosser, *Curr. Protoc. Immunol.*, 2008, **83**, 14.1.1-14.1.14

[S2] E. H. Byun, W. S. Kim, J. S. Kim, I. D. Jung, Y. M. Park, H. J. Kim, and S. J. Shin, *FASEB J.*, 2012, **26**, 2695-2711.