The influence of modified molecular (D/L-serine) chirality on the theragnostic of PAMAM-based nanomedicine for acute kidney injury

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1. Materials and methods

Synthesis of NPSH [1]

The $POCl_3$ (18.5 mL, 198 mmol) and CH_2Cl_2 (17.5 mL) solutions were added to an ice solution of 20 mL DMF (258 mmol) and 20 mL CH_2Cl_2. After completing the addition, cyclohexanone (5 g, 50 mmol) was slowly added through a syringe; the mixture was refluxed for 3 h. After the reaction is over, cool to room temperature, the mixture was dropped into 150 mL ice water mixture slowly, standing overnight. Many yellow crystals (2-Chloro-1-formyl-3-hydroxyl methyl cyclohexene) were appeared, filtered, and washed with CH_2Cl_2 and distilled water. 2-Chloro-1-formyl-3-hydroxyl methyl cyclohexene, 1,2,3,3-tetramethyl-3H-indoliodide (2.67 g, 4.05 mmol) and 70 mg sodium acetate were dissolved in 8 mL acetic anhydride, and the mixture was stirred at 60 °C for 3h. After the reaction, it was cooled to room temperature, filtered, and washed with $NaHCO_3$ until there is no bubble, and then washed with water three times. The golden-green powder (1,1',3,3',3'-hexamethylindole iodide dicarbocyan) was obtained by drying. Para-bromodescalcinol (1.88 g, 10 mmol) and 2 mL triethylamine were added into a three-neck flask containing 10

mL DMF. For 10 min reaction under the protection of N_2, 50 °C. 1',3,3',3' -hexamethylindole iodide dicarbocyan (3.0 g, 5 mmol) was dissolved in 10 mL DMF and added into the reactor through a syringe. The temperature was raised to 55 °C for 6 h; the reaction solution was poured into ice water and extracted with CH_2Cl_2. Anhydrous Na_2SO_4 was added to the organic phase for drying for 4 h. Then the sample was dried and mixed by spinning, CH_2Cl_2:EA = 1:1, and triethylamine development agent was added for column chromatography. The product of the above step (0.59 g, 1 mmol) and CS_2CO_3 (1.63 g, 5 mmol) was dissolved in 20 mL CH_2Cl_2 and stirred for 30 min under the protection of N_2. 4(Bromomethyl) phenylboric acid (0.426 g and 2 mmol) were dissolved in 10 mL THF, it was added into the above reaction solution through a syringe and then heated to 50 °C for 3 h. After the reaction, the organic solvent is removed, and the developing agent CH_2Cl_2:EtOH = 10:1 was used for column chromatography to obtain the blue solid powder NPSH. ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm): 8.73-8.67 (m, J = 8.69Hz, 1H), 7.84 (d, J = 7.83Hz, 1H), 7.74-7.69 (m, J = 7.71Hz, 3H), 7.58-7.50 (m, J = 7.54Hz, 5H), 7.58-7.24-7.13 (m, J =7.19Hz, 2H), 6.57-6.53 (d, J = 6.55Hz, 1H), 5.39 (s, 2H), 3.89 (s, 3H), 3.34 (s, 2H), 2.77 (t, J= 2.75Hz, 2H), 2.72 (t, J = 2.71Hz, 2H), 1.93 (t, J = 1.91Hz, 2H), 1.82(s, 6 H). MS (ESI): calcd. For $C_{33}H_{32}BBrNO_4^+$ 596.1, found 612.3 [M+H_2O] (Figure S8 and S9).

2. Fabrication of CO Probe 3-benzothiazolyl-7-hydroxycoumarin (BTHC) [2]

2,4-dihydroxy benzaldehyde (345.3 mg, 2.5 mmol) and benzothiazole-2-acetonitrile (435.55 mg, 2.55 mmol) were dissolved in 5 mL ethanol, and 5 drops of piperidine were added. The mixture was stirred at room temperature for 6 h. At the end of the reaction, the yellow solid was treated with 10% hydrochloric acid; the suspension was stirred at 130 °C for 3 h. The yellow residue was collected after extraction and filtration, washed with water dried in a vacuum. Finally, the compound was obtained by silica gel column chromatography (CH_2Cl_2:EtOH = 5:1, v/v). Allyl chloroformate (180 mg, 1.5 mmol) and ET_3N (150 μL) were added to dichloromethane (5 mL) solution containing 47 mg, 0.5 mmol, and stirred at room temperature until the reaction was complete. The resulting solution was washed three times with distilled water, and the dichloromethane phase was dried on Na_2SO_4. After the organic solvent was filtered and removed, the yellow-green solid product

was formed, further purified by column chromatography with EA:PE= 1:5 developing agent to obtain pure yellow product BHTC. ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm): 8.73-8.67 (m, J = 8.69Hz, 1H), 7.84 (d, J = 7.83Hz, 1H), 7.74-7.69 (m, J = 7.71Hz, 3H), 7.58-7.50 (m, J = 7.54Hz, 5H), 7.58-7.24-7.13 (m, J =7.19Hz, 2H), 6.57-6.53 (d, J = 6.55Hz, 1H), 5.39 (s, 2H), 3.89 (s, 3H), 3.34 (s, 2H), 2.77 (t, J= 2.75Hz, 2H), 2.72 (t, J = 2.71Hz, 2H), 1.93 (t, J = 1.91Hz, 2H), 1.82(s, 6 H). MS (ESI): calcd. For $C_{33}H_{32}BBrNO_4^+$ 596.1, found 612.3 [M+H_2O] (Figure S10 and S11).

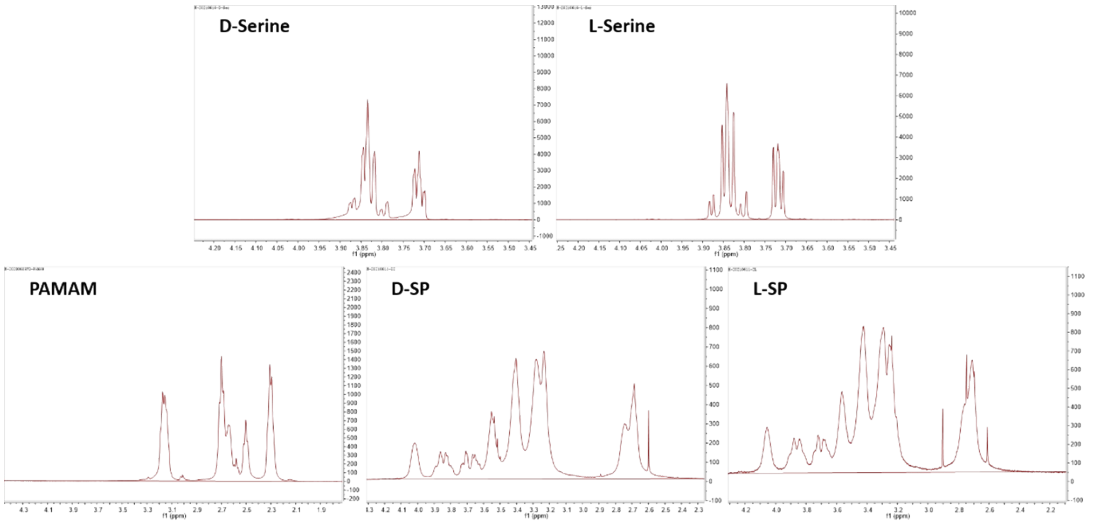

Figure S1. Nuclear magnetic resonance spectrum of D-Serine, L-Serine, PAMAM, D-SP and L-SP.

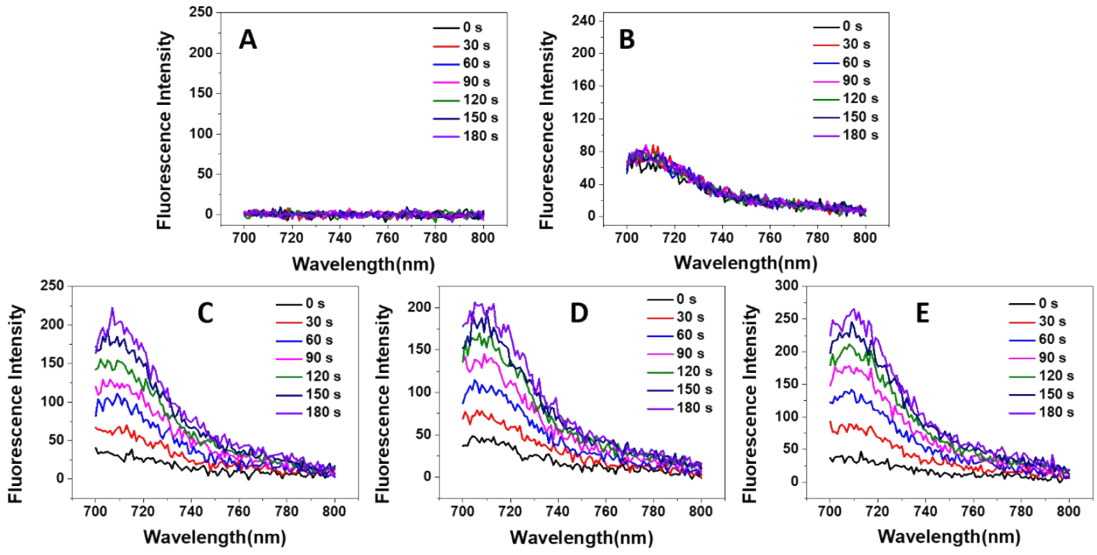

Figure S2. (A) Control group, (B) NPSH, and (C)L-SPH, (D) D-SPH and (E) NPSH respond to H_2O_2 to produce fluorescence changes in solution.

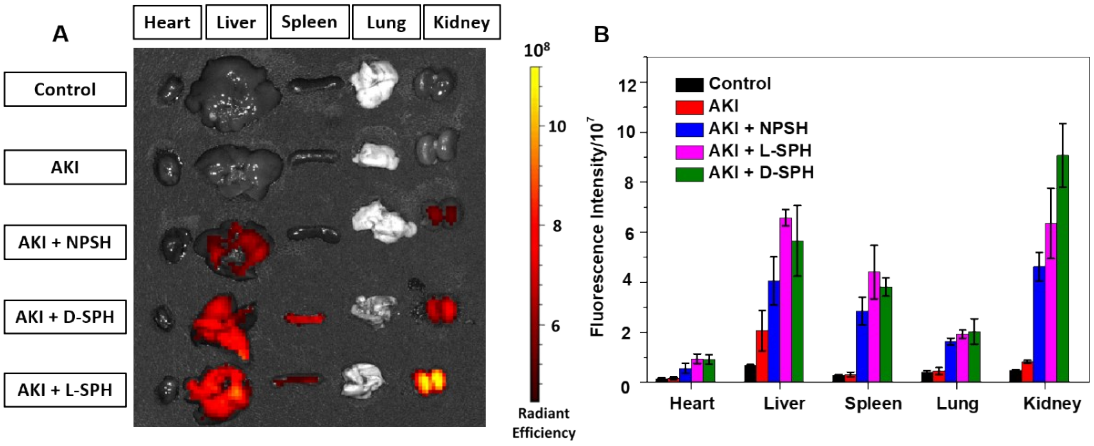

Figure S3. (A) Fluorescence imaging and (B) fluorescence intensity comparison of kidney and other organs in different mice groups.

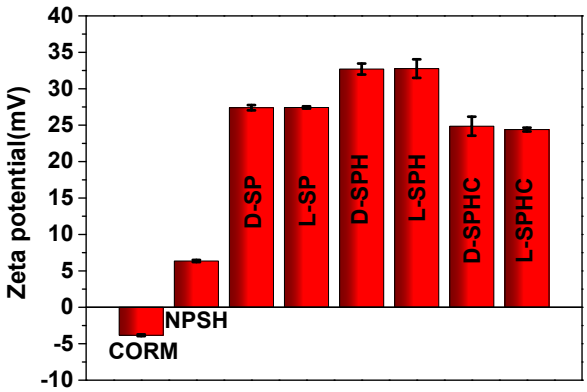

Figure S4. Zeta potentials of CORM, NPSH, D-SP, L-SP, D-SPH, L-SPH, D-SPHC, L-SPHC.

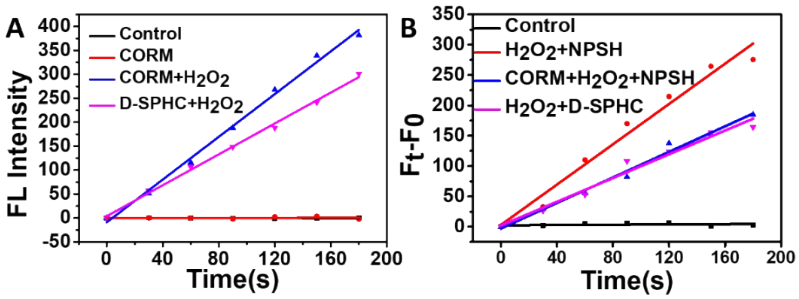

Figure S5. (A) BHTC-CO probe detects CO production in CORM, CORM with H_2O_2, D-SPHC with H_2O_2 group in solution; (B) Fluorescence intensity in control, H_2O_2 detected by

NPSH, CORM with H_2O_2 detected by NPSH, D-SPHC with H_2O_2 group in solution.

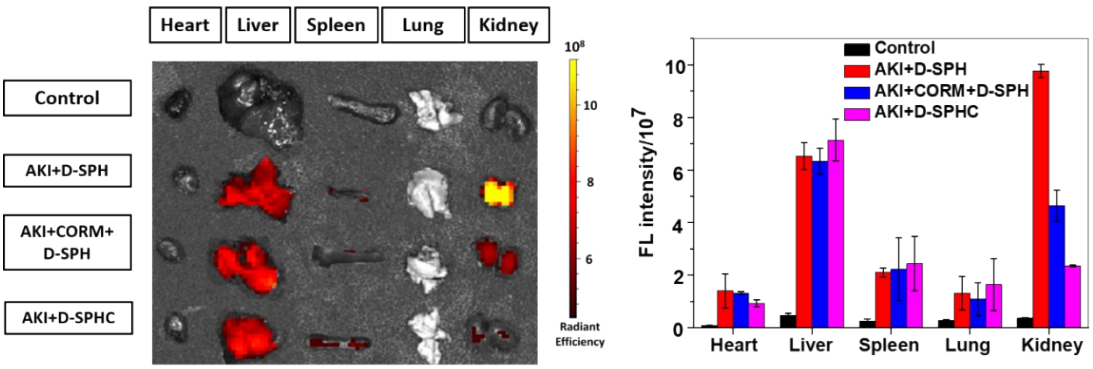

Figure S6. Fluorescence imaging and fluorescence intensity comparison of kidney and other organs in different mice groups.

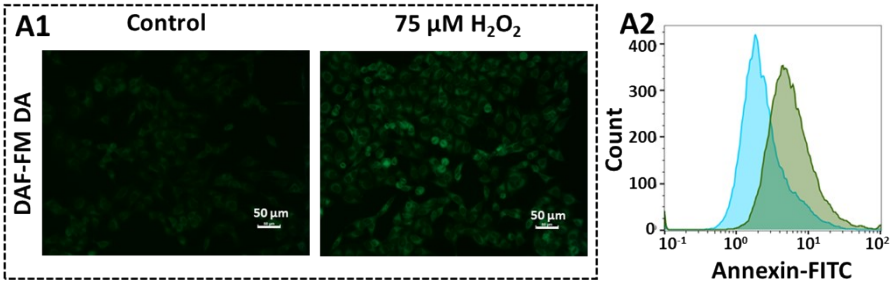

Figure S7. (A) DAF-FM DA fluorescence images (1) and intensity changes by flow cytometry detection of in control and H_2O_2 treated Raw 264.7 cells.

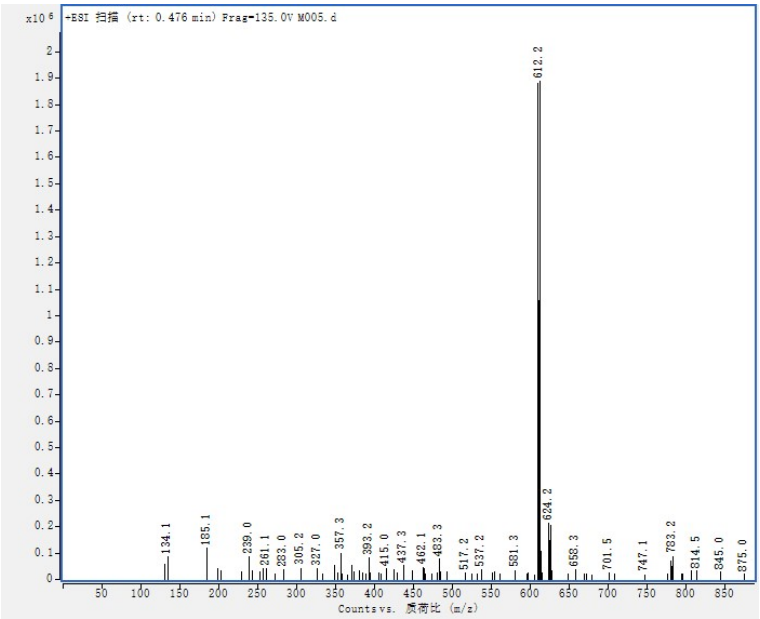

Figure S8. Mass spectrogram of NPSH. (A) DAF-FM DA fluorescence images (1) and intensity changes by flow cytometry detection of in control and H2O2 treated Raw 264.7 cells.

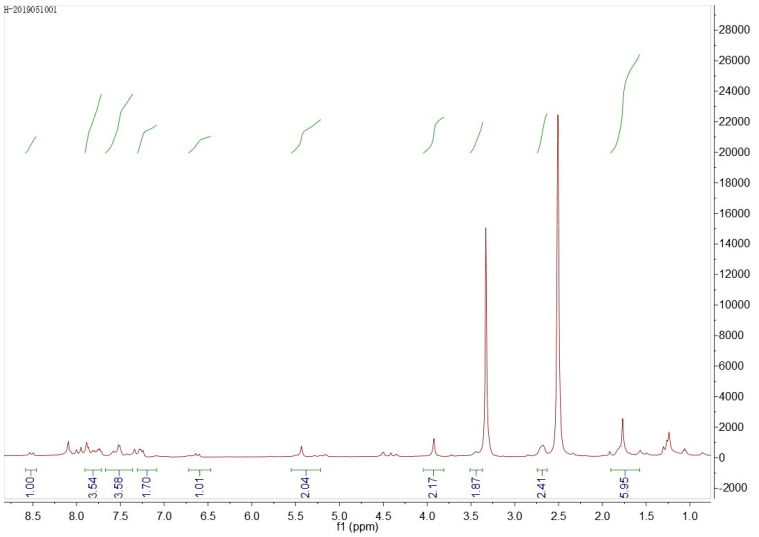

Figure S9. Nuclear magnetic resonance spectrum of NPSH.

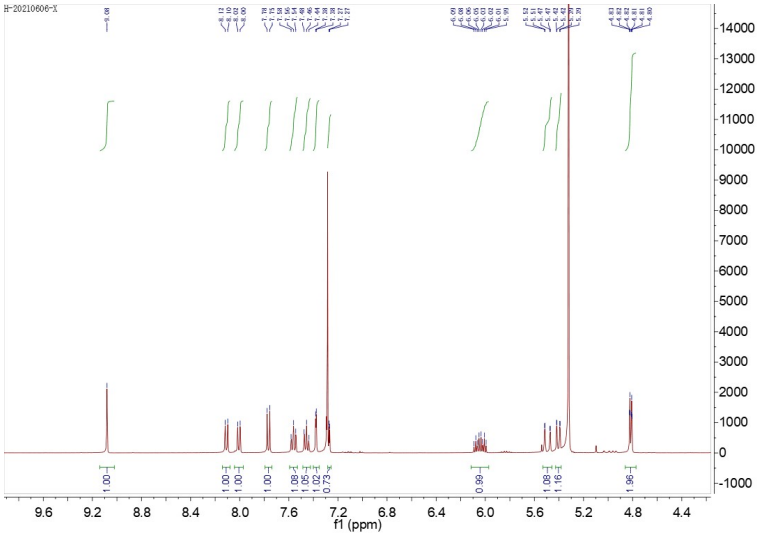

Figure S10. Mass spectrogram of BHTC.

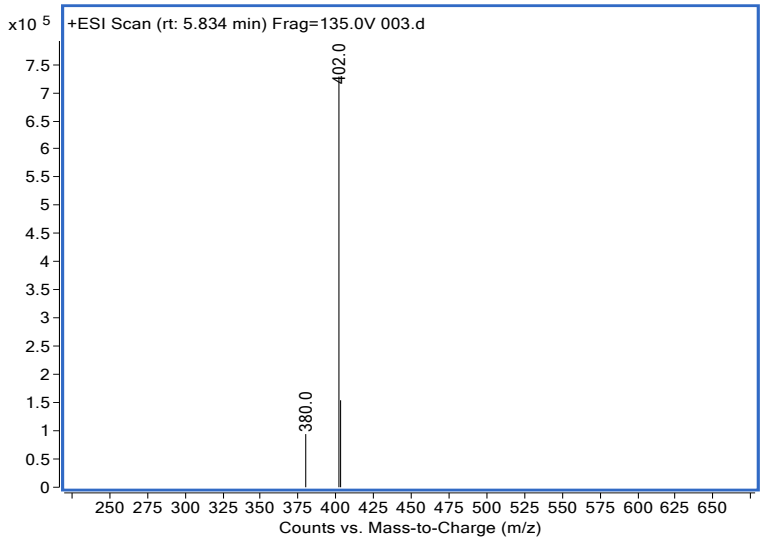

Figure S11. Mass spectrogram of BHTC.

References

1 H.W. Liu, X.X. Hu, K. Li, Y. Liu, Q. Rong, L. Zhu, L. Yuan, F.L. Qu, X.B. Zhang, W. Tan, A mitochondrial-targeted prodrug for NIR imaging guided and synergetic NIR photodynamic-chemo cancer therapy, Chem. Sci. 2017, 8, 7689-7695.

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