Supplementary Information for

Global identification of cellular targets of multi-molecules system by photochemically-induced coupling reaction

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Materials and methods

1. Chemicals and reagents

$FeCl_3 \cdot 6H_2O$ was purchased from ANFEL Laboratory Technologies (Shanghai, China), sodium citrate (Na_3Cit), 4,4'-Dihydroxybenzophenone (DHBP) and ethylene glycol (EG) were purchased from Energy Chemical (Shanghai, China). 2-isocyanatoethyl 2,6-diisocyanatohexanoate was purchased from BioRuler (Danbury CT, USA), sodium acetate ($NaAc \cdot 3H_2O$) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetone and methanol were purchased from Beijing Chemical Works (Beijing, China).

Medicinal plant *Dendranthema indicum* (DI) was obtained from Beijing Tong Ren Tang (Chinese medicine shop, Beijing, China). A collection of 33 natural compounds (purity > 98%) including cynarin (1), hesperetin (2), myricetin (3), hesperidin (4), neochlorogenic acid (5), sesamin (6), carotenoid (7), fargesin (8), hyperoside (9), quercetin (10), acacetin (11), 1,5-dicaffeoylquinic acid (12), luteolin (13), ferulic acid (14), kaempferol (15), chlorogenic acid (16), scutellarein (17), rutinum (18), apigenin 7,4'-dimethyl ether (19), 2',4'-dihydroxychalcone (20), 7-hydroxy-2,3-dihydro-2-flaconoid (21), chrysophanol (22), 3,4-dicaffeoylquinic acid (23), eupatilin (24), isochlorogenic acid C (25), isochlorogenic acid B (26), ursolic acid (27), gallic acid

(28), diosmetin-7-O-β-D-glucopyranoside (29), cyanidin-3-O-glucoside (30), isochlorogenic acid A (31), hispidulin (32) and apigenin-7-O-glucoside (33) were obtained from TargetMol (Shanghai, China) and affirmed by H^1NMR and MS data. The purity of the compounds was measured by HPLC to be >98%. The mouse recombinant UBE2I, PRDX6, RHOA, PRKCH, CREBBP and EP300 protein were expressed in *Escherichia coli*, respectively. Lipopolysaccharide (LPS) from *Escherichia coli* O55:B5 was obtained from Sigma-Aldrich (St. Louis, MO, USA). 3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide (MTT) was obtained from Sigma Chemical (Santa Clara, CA, USA). High glucose Dulbecco's modified eagle medium (DMEM, 4.5 g/L glucose), antibiotics and trypsin were from Macgene (Beijing, China). Fetal bovine serum (FBS) was from PAN-Biotech (Logan, UT, USA).

2. Preparation of sulfhydryl bound Fe_3O_4 NPs

$FeCl_3 \cdot 6H_2O$ (0.325 g) and Na_3Cit (0.20 g) were dissolved in ethylene glycol (20 mL). Then, $NaAc \cdot 3H_2O$ (1.20 g) was added and the mixture was stirred until full dissolution in a Teflon-lined stainless-steel autoclave. The autoclave was heated at 200 °C for 8 h. The product (15.0 mg) was washed with ethanol and dispersed in DMSO (5 mL). Then, racemic-2,3-dimercaptosuccinic acid (15 mg) was added and shaken at 50 °C for 1.2 h. Finally, the sulfhydryl-bound Fe_3O_4 NPs were prepared and washed with acetone.

3. Preparation of DHBP-bound Fe_3O_4 NPs

The DHBP-bound Fe_3O_4 NPs were prepared by click-chemistry reaction. Briefly, sulfhydryl loaded Fe_3O_4 NPs (15.0 mg) was dissolved in acetone (50 mL). Then, ethyl ester L-Lysine triisocyanate (44.5 mg) and 4,4'-Dihydroxybenzophenone (42.8 mg) were added with stirring at 200 revolutions per minute for 2 h. The DHBP-bound Fe_3O_4 NPs were washed with methanol for five times to remove impurities.

4. Chemicals crosslinked onto DHBP-bound Fe_3O_4 NPs

4,4'-Dihydroxybenzophenone-bound Fe_3O_4 NPs (5 mg) and chemical extract from *Dendranthema indicum* (DI, 5 mg) were mixed in 20 mL methanol. The mixture was irradiated under UV (254−365 nm) by a mercury lamp (power: 200 W, light intensity: 3.3 $W \cdot cm^{-2}$) at 25 °C for 1h. Then, the carbonyl groups of diphenylketone on Fe_3O_4 NPs surface were activated, and crosslinked with the chemical extract from DI.

5. Field-emission scanning electron microscope (FE-SEM) analysis

Microstructure of the nanoparticles was evaluated by field-emission scanning electron microscope (Merlin Compact, ZEISS, Jena, Germany). The Fe_3O_4 NPs were fixed on the silicon wafer. Then they were photographed under a field-emission scanning electron microscope at an accelerating voltage of 5 kV at magnifications of 500×, 1000×, 2000× and 5000 ×.

6. Fourier transform infrared (FT-IR) spectroscopy analysis

FT-IR (Thermo Scientific Nicolet iN5, Waltham, MA, USA) was applied for the surface properties analysis. The dried samples were powdered finely using an agate mortar and pestle. 2 mg sample was mixed with 200 mg KBr. Finally, each sample was compressed at a pressure utilizing a hydraulic pellet press to engender a thin KBr pellet for the analysis. Then, the pellet was scanned with an FT-IR spectrometer in the frequency range of 400 - 4000 cm^{-1}.

7. Transmission electron microscope (TEM) analysis

Samples for TEM analysis were prepared by spreading a drop of the as-synthesized Fe_3O_4 nanoparticle dilute dispersion on copper grids coated with a carbon film followed by evaporation under ambient conditions. The morphology and structure of the prepared Fe_3O_4 NPs were characterized by TEM at an accelerating voltage of 80 kV (Tecnai G2 F20, FEI, Hillsboro, OR, USA).

8. Cell culture

Murine BV-2 microglial cell line was purchased from the Cell Bank of Peking Union Medical College (Beijing, China). The cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 mg/mL streptomycin at 37 °C in a 5% CO_2 incubator under 95% absolute humidity.

9. Cellular target protein pull-down

DI extract-crosslinked NPs were washed 6 times with washing buffer (50 mM HEPES, 30 mM NaCl, 1 mM EDTA, 2.5 mM EGTA, 0.1% Tween20, pH7.5) and then incubated with BV-2 cell lysates for 12 h under constant rotation at 4 °C. Meanwhile, an excess amount of DI extract for competition was added into lysates. Target proteins

captured from the cell lysates by DI extract-crosslinked NPs were eluted and subjected to tryptic digestion. After that, the proteins were analyzed by quantitative MS.

10. MS/MS analysis of target proteins

The trypsin-digested samples were first filtered through a 0.22 μm micro-pore membrane and then subjected to liquid chromatography coupled with a LTQ Velos pro mass spectrometer (Thermo Fisher Scientific, MA, USA). The Captrap Peptide column (20 μL/min) was used to load the peptide solution (10 μL), and separation of the analytes were achieved on a RP-C18AQ column (100 μm id×15 cm, Michrom Bioresources, USA), with a column oven temperature of 35 °C. The electro spray voltage was operated at 1.8 kV.

11. Surface plasmon resonance (SPR) analysis

Interactions between 33 active ingredients of DI and 6 target proteins were quantitatively analyzed using the Biacore T200 system (GE Healthcare, Boston, USA). The purified recombinant UBE2I, PRDX6, RHOA, PRKCH, CREBBP and EP300 proteins (500 μg/mL) were respectively dissolved in running buffer (Tris-HCl 50 mM, pH 7.2, 100 mM KCl) and immobilized on a carboxymethylated 5 sensor chip using a standard amine coupling method in 10 mM sodium acetate at pH 4.5. Ligand solutions were injected as analytes. Response unit (RU) signal was used to preliminarily represent binding profile of DI active constituents to their bound target proteins. For kinetic analysis of the interactions between myricetin and 6 target proteins, gradient concentrations of myricetin (0.78 to 200 μM) in the running buffer were injected as analytes. Data were analyzed with the Biacore evaluation software (T200 Version 2.0). The kinetic parameters of K_a, K_d and K_D were derived by fitting to a 1:1 Langmuir binding model.

12. Molecular docking

The crystal structure of UBE2I (PDB code: 5F6E), PRDX6 (PDB code: 5B6M), RHOA (PDB code: 6BCA), PRKCH (PDB code: 2FK9), CREBBP (PDB code: 5I89) and EP300 (PDB code: 5NU5) were obtained from the Protein Data Bank of RCSB. Docking simulations were carried out by SYBYL-X 2.0 molecular modeling package (Tripos International, St Louis, MO, USA). The standard parameters of SYBYL-X

were used to plot the ligand molecules, and then the Tripos force field was used to minimize the energy in 1000 steps to calculate the Gasteiger-Huckel charges. Protein receptors were prepared by deleting the ligand and all water molecules. Then, myricetin was docked into the mode of six proteins using Surflex-Dock of SYBYL-X 2.0. The hydrogen bond is represented by a dotted line. Structural images were prepared with PyMOL (Schrödinger, LLC, Palo Alto, CA, USA).

13. Cell viability assay

Cell viability was detected via colorimetric MTT assay. After treatment, culture medium was replaced with MTT solution (0.5 mg/mL) and incubated for 4 h. Then, medium was replaced with 100 μL of dimethyl sulfoxide (DMSO). Absorbance was detected at 570 nm using Infinite F50 Absorbance Microplate Reader (Tecan, Mannedorf, Switzerland). Cell viabilities in different groups were normalized to blank group which was set as 100%.

14. Nitrite oxide analysis

BV-2 cells were treated with 33 compounds (1, 10 and 50 μM) with or without LPS for 24 h. Then, the supernatants were used to determine the production of nitrite oxide (NO) with a nitric oxide analysis kit (Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China).

15. Statistical analysis

All experiments were carried out at least three times in triplicate. Statistical analyses were performed by using one-way analysis of variance (ANOVA) with GraphPad Prism 8.0 software. Multiple comparisons were performed by comparing the mean of each column with the mean of every other column. All data were expressed as the means ± S.D. $P < 0.05$ was considered to indicate statistical significance.