Supporting Information
For

Redox Responsive Pd (II) Templated Rotaxane Nanovalve Capped Mesoporous Silica Nanoparticles: A Folic Acid Mediated Biocompatible Cancer-targeted Drug Delivery System

Srivardhan Reddy Gayam, and Shu–Pao Wu*

Department of Applied Chemistry, National Chiao Tung University, Hsinchu, 30010, Taiwan, Republic of China

E–mail: spwu@mail.nctu.edu.tw
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Scheme S1. Synthesis schemes for (A) synthesis of S-(2-aminoethylthio)-2-thiopyridine hydrochloride (1); (B) synthesis of 2-((3-(2-azidoethoxy)phenoxy)methyl)-6-(bromomethyl)pyridine (4); (C) synthesis of macrocycle Pd-tridentate ligand; (D) synthesis of alkyne-functionalized folic acid.
Synthesis of S-(2-aminoethylthio)-2-thiopyridine hydrochloride (1): S-(2-Aminoethylthio)-2-thiopyridine hydrochloride was synthesized based on a literature reported by Yon W. Ebright et al.1 but with further modification. Typically, thiopyridyl disulfide (2.2 g, 10 mmol) was dissolved in MeOH (10 mL) solution containing acetic acid (0.4 mL). MeOH solution (10 mL) containing 2-aminoethylthiol hydrochloride (570 mg, 5.04 mmol) was added drop wise into the above solution for 30 min. After stirring for 60 h, solvent was removed under reduced pressure to yield a yellow oily product. The yellow oily product was washed with cold diethyl ether (50 mL) for two times and then dissolved in MeOH (10 mL). The solution was added to chilled (−30 °C) diethyl ether (200 mL). Yellow precipitate started appearing, and this solution was kept at −30 °C for 2 h for precipitate to settle down. Yellow precipitates were collected by vacuum filtration. Repeat the precipitation experiment to obtain a pure product. 1H NMR (300 MHz, CDCl3, δ): 9.4 (s, 2H), 8.71 (d, J= 3.3 Hz, 1H), 7.63 (t, J= 5.1 Hz, 1H), 7.38 (d, J = 6.0 Hz, 1H); 7.24 (t, J= 4.5 Hz, 1H); 3.41 (d, J = 3.9 Hz, 2H); 3.33 (d, J = 4.2 Hz, 2H); 13C NMR (75 MHz, DMSO-d6, δ): 158.6, 150.3, 138.4, 122.1, 120.5, 38.2, 35.2; MS (ESI+); m/z calculated for C7H10N2S2 is 186.03; found [M+H]+ =187.2

Synthesis of 3-(2-bromoethoxy)phenol (2): 1-(Benzyloxy)-3-(2-bromoethoxy)benzene (1.625 g, 5.29 mmol) was added to dry THF/EtOH (8/2, v/v, 25 ml) solvent. (Prior to the experiment, the round bottomed flask was evacuated by 3 freeze-thaw-pump cycle). To the above solution, 10% Pd/C (0.1625 g, 10 w %) was added carefully and then further evacuated by a freeze-thaw-pump cycle. H2 was applied through balloon and the mixture was stirred at room temperature for 10 h. The solution was filtered through celite bed and then washed with 40 mL THF. The organic layer was dried over MgSO4 and the solvent was evaporated under reduced pressure to give pale brown liquid (980 mg, 9.6 mmol, 89%). 1H NMR (300 MHz, CDCl3, δ): 7.13 (t, J = 8.0 Hz, 1H), 6.51-6.42 (m, 3H), 4.25 (t, J= 6.3 Hz, 2H), 3.63 (t, J = 6.3 Hz, 2H); 13C NMR (75 MHz, CDCl3, δ): 159.1, 156.4, 130.2, 108.6, 107.0, 102.4, 67.7, 29.1; MS (ESI+); m/z calculated for C8H10BrO2 is 217.05; found [M+H]+ = 218.06.

Synthesis of 3-(2-azidoethoxy)phenol (3): To a solution of 3-(2-bromoethoxy)phenol (2) (670 mg, 3.07 mmol) in DMF (15 mL), NaN3 (600 mg, 9.21 mmol) was added and the mixture was stirred at 60 °C for 10 h. The mixture was cool down to room temperature and poured into Et2O (150 mL) and H2O (150 mL). The organic phase was washed with H2O (2
x 200 ml) and followed by brine solution (2 x 75 ml). The washed organic phase was dried over (MgSO₄) and the solvent was removed under reduced pressure. The crude residue was purified by column chromatography (hexane/ethyl acetate, 9/1 to 6/4) to give compound 3 as a brown liquid (464 mg, 7.8 mmol, 84%). ¹H NMR (300 MHz, CDCl₃, δ): 7.17 (t, J = 8.1 Hz, 1H), 6.54-6.46 (m, 3H), 5.10 (s, br,1H), 4.10 (t, J = 4.95 Hz, 2H), 3.57 (t, J = 4.95 Hz, 2H); ¹³C NMR (75 MHz, CDCl₃, δ): 159.1, 156.5, 130.1, 108.4, 106.6, 102.1, 66.6, 49.8; MS (ESI +): m/z calculated for C₈H₃N₃O₂ is 179.18; found [M+H]+ =180.

Synthesis of 2-((3-(2-azidoethoxy)phenoxy)methyl)-6-(bromomethyl)pyridine (4): To a solution of 3-(2-azidoethoxy)phenol (3) (300 mg, 1.67 mmol, 1.0 equiv) in acetonitrile (10 ml) K₂CO₃ (2.8 g, 20.1 mmol, 12.0 equiv) was added. The mixture was stirred for 20 min at 60 ºC. 2,6-bis(Bromomethyl)pyridine (1.35 g, 5.10 mmol, 3.05 equiv) in acetonitrile (20 mL) was added slowly over a period of 10 min and the suspension was stirred under reflux for 24 h. After cooling, the mixture was filtrated and the residual solid was washed with EtOAc. The filtrate was evaporated under reduced pressure and the crude residue was purified by column chromatography (hexane/ethyl acetate, 9/1) to give compound 4 as a pink solid (322 mg, 0.89 mmol, 53%). ¹H NMR (300 MHz, CDCl₃, δ): 7.73 (t, J = 7.8 Hz, 1H), 7.45 (d, J = 7.7 Hz, 1H), 7.36 (d, J = 7.7 Hz, 1H), 7.18 (t, J = 8.1 Hz, 1H), 6.63-6.53 (m, 3H) 5.18 (s, 2H), 4.56 (s, 2H), 4.12 (t, J = 5.0 Hz, 2H), 3.57 (t, J = 5.0 Hz); ¹³C NMR (75 MHz, CDCl₃, δ): 159.3, 159.2, 157.0, 156.0, 137.7, 129.9, 122.2, 120.3, 107.4, 107.2, 101.8, 70.2, 66.7, 49.9, 33.5; MS (ESI+): m/z calculated for C₁₅H₁₅BrN₄O₂ is 362.04; found [M+H]+ = 363.2.

Synthesis of macrocyclic-Pd tridentate ligand: Macrocyclic-Pd tridentate ligand was prepared based on the literature reported. To a solution of 1,10-bis[p-aminomethyl]phenoxy]decane (1.25 g, 3.725 mmol) in anhydrous dichloromethane (500 mL), triethylamine (0.97 g, 9.7 mmol) was added at 0 ºC under nitrogen. A solution of 2,6-pyridinedicarbonyl dichloride (0.9 g, 4.0 mmol) in anhydrous dichloromethane (20 mL) was slowly added drop wise over 2 h, while keeping the solution at 0 ºC. The solution was then warmed to room temperature and stirred for 18 h. The solvent was removed under reduced pressure. The crude residue was purified by column chromatography (CH₂Cl₂/ethyl acetate, 1:1) and recrystallized from acetonitrile to yield colorless crystals (1.1 g, yield = 49%). Dissolved colorless crystals (0.52 g, 1.00 mmol) in anhydrous acetonitrile (15 mL), palladium (II) acetate (0.25 g, 1.00 mmol) was added and the reaction stirred at room
temperature for 1 h under an atmosphere of nitrogen. The resulting precipitate was filtered, washed with acetonitrile (25 mL) and dried under suction to yield a yellow solid (0.57 g, yield = 86%), (NMR data is only for macrocycle, before complexation with palladium(II) acetate).  

\[ \text{H NMR (300 MHz, CD}_2\text{Cl}_2, \delta): 8.37 (d, J = 7.8 Hz, 2H), 8.03 (t, 1H, J = 7.8 Hz), 7.9 (br, 2H), 7.21 (d, J = 8.6 Hz), 6.83 (d, 4H, J = 8.6 Hz), 4.62 (d, 4H, J = 6.1 Hz), 3.95 (t, 4H, J = 6.3 Hz), 1.76 (m, 4H), 1.29-1.47 (m, 12H); \]  
\[ \text{C NMR (75 MHz, CD}_2\text{Cl}_2, \delta): 25.8, 28.4, 28.8, 29.0, 43.2, 67.8, 115.1, 125.6, 129.5, 130.1, 139.2, 149.1, 158.9, 163.6; MS (ESI+); m/z calculated for C\text{31}H\text{35}N\text{3}O\text{4}Pd 619.17; found [MH-CH_3CN]^+ = 620. \]

**Synthesis of alkyne-functionalized folic acid:** The synthesis of alkyne-functionalized folic acid was prepared based on the literature report. Folic acid (1.0 g, 0.22 mmol) was dissolved in DMF (10 mL) and cooled in a water/ice bath. N-Hydroxysuccinimide (260 mg, 0.25 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (440 mg, 0.25 mmol) were added, and the mixture was stirred in the ice bath for 30 min to give a white precipitate. A solution of propargylamine (124 mg, 2.25 mmol) in DMF (5.0 mL) was added, and the mixture was allowed to reach room temperature and stirred for 24 h. The reaction mixture was poured into water (100 mL) and stirred for 30 min to form precipitates. The orange-yellow precipitates were filtered, washed with acetone, and dried under vacuum for 6 h to yield 0.97 g of product (yield = 89%).  

\[ \text{H NMR (300 MHz, DMSO-}d_6, \delta): 11.53 (br, 1 H), 8.64 (s, 1 H), 8.3 (t, 1 H), 8.1 (d, 1 H), 7.67(d, 2 H), 6.95 (br, s, 2 H), 6.65 (d, 2 H), 4.49 (d, 2 H), 4.34 (m, 1 H), 3.82 (m, 2 H), 2.51 (s, 1 H), 2.51 (br, 1 H), 2.19 (m, 2 H), 2.01 (m, 1 H), 1.98 (m, 1 H); \]  
\[ \text{C NMR (75 MHz, DMSO-}d_6, \delta): 27.8, 28.3, 28.5, 31.2, 32.3, 46.4, 53.4, 73.2, 81.67, 111.6, 121.8, 128.4, 129.6, 149.0, 151.2, 154.3, 166.7, 171.9, 172.1; MS (ESI+); m/z calculated for C\text{22}H\text{22}N\text{8}O\text{4} 478.17; found [M+H]^+ = 479.08 and m/z calculated for C\text{25}H\text{25}N\text{9}O\text{4} is 515.15; found [M+H]^+ = 516. \]
**Figure S1.** Mesoporous silica nanoparticle size analysis (a) DLS particle size analysis of MSNP–SS–N$_3$ and (b) scanning electron microscope (SEM) image of MSNP–SS–N$_3$

**Figure S2.** Powder XRD pattern of (a) MSNP–SH, (b) MSNP–SS–NH$_2$, and (c) MSPN–SS–N$_3$ and with illustration of lattice interplanar spacing of mesopore structure.

Braggs law: $n\lambda=2d \sin \theta$

$\lambda=1.5406 \ A^0$

$n=1$

Lattice interplanar spacing: $d = \frac{n\lambda}{2\sin \theta}$
Figure S3. BET isotherm and BJH pore size distribution of (a) MSNP–SH, (b) MSNP–SS–NH₂, and (c) MSPN–SS–N₃, and (d) fluorescein loaded MSPN–SS–N₃.
Figure S4. (a) TEM and (b) HR-TEM images of MSPN–SS–FA

Figure S5. Zeta potential values of different mesoporous silica nanoparticles during the functionalization
Figure S6. X-ray photoelectron spectroscopy (XPS) of MSNP–SS–FA
From the TGA data, the weight loss of MSNPs, MSNP–SH, MSNP–SS–FA and fluorescein loaded MSNP–SS–FA were approximately estimated as 6.7 %, 9.6 %, 13.3 %, 15.2 % respectively. The 6.6 % difference in weight loss from MSNPs to MSNP–SS–FA indicates the weight loss of rotaxane nanovalves on the surface of MSNPs and the surface density of rotaxane nanovalves is calculated to be 66 mg g\(^{-1}\). The loading amount of fluorescein was 19 mg g\(^{-1}\), calculated by the difference of weight loss between (d) fluorescein loaded MSNP–SS–FA and (c) MSNP–SS–FA.
Figure S8. Fluorescence spectra of fluorescein (——) before loading and (-----) after loading in MSNP–SS–N\textsubscript{3} with 3:1 weight ratio in PBS buffer.

Figure S9. Fluorescence microscopic images of HeLa cells and HEK-293 cells after being treated with 25 μg mL\textsuperscript{-1} of fluorescein-loaded MSNP–SS–FA for 4h.
Reference:


