Supporting Information

Theranostic Nanoscale Coordination Polymers for Magnetic Resonance Imaging and Bisphosphonate Delivery

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1. **Materials and Methods.** All of the starting materials were purchased from Aldrich and Fisher, unless otherwise noted, and used without further purification. 1,2-dioleoyl-sn-glycero-3-phosphate sodium salt (DOPA), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)2000] (DSPE-PEG_{2k}) were purchased from Avanti Polar Lipids. Cell culture supplies fetal bovine serum (Sigma), RPMI-1640 growth medium (Gibco), penicillin-streptomycin (Sigma), and phosphate buffered saline (Gibco) were purchased from the Cell Culture Facility in the Lineberger Cancer Center at UNC-Chapel Hill. Microwave reactions were carried out in a CEM Discovery microwave. The ^{1}H NMR spectra were recorded on a Bruker NMR 400 DRX Spectrometer at 400 MHz and referenced to the proton resonance resulting from incomplete deuteration of deuterated chloroform or DMSO. UV-Vis absorption spectra were obtained using a Shimadzu UV-2401PC UV-Vis Spectrophotometer. Thermogravimetric analyses (TGA) were performed using a Shimadzu TGA-50 equipped with a platinum pan. A Hitachi 4700 field emission scanning electron microscope (SEM) and a JEM 100CX-II transmission electron microscope were used to determine particle size and morphology. SEM images of the nanoparticles were taken on glass substrate. A Cressington 108 Auto Sputter Coater equipped with a Au/Pd (80/20) target and MTM-10 thickness monitor was used to coat the sample with approximately 5 nm of conductive layer before taking SEM images. Size and zeta potential data were collected with a Malvern Zetasizer Nano Zs. A Varian 820-MS Inductively Coupled Plasma-Mass Spectrometer was used to determine Mn concentration. Samples were introduced via a concentric glass nebulizer with a free aspiration rate of 0.4 mL/min, a Peltier-cooled double pass glass spraychamber, and a quartz torch. A peristaltic pump carried samples from a SPS3 autosampler (Varian) to the nebulizer. All standards and samples were in 2% HNO_3, prepared with milliQ water. The matrix solution for MALDI-TOF analysis was prepared by dissolving 2,5 dihydroxybenzoic acid (10 mg, 0.065 mmol) in acetonitrile (1 mL). DSPE-PEG-AA (1 ng, 0.340 pmol) was dissolved in 300 μL of the matrix solution. After vortexing, the sample/matrix solution (1 μL) was spotted onto a Bruker MTP 384 ground steel plate and dried at room temperature. The mass spectrometry analysis was carried out on a Bruker Ultraflextreme MALDI-TOF MS using a positive reflective ion mode at a mass range of 700-4500 Da.

2. **Synthesis of DSPE-PEG_{2k}-AA.**

4-methoxybenzoic acid (3 g, 20 mmol) in 10 mL thionyl chloride was refluxed for 2 h. Remaining thionyl chloride was removed in vacuum to give 4-methoxybenzoyl chloride. Under Ar gas, a solution of 4-methoxybenzoyl chloride (1.000 g, 5.862 mmol) in 10 mL CH_2Cl_2 was slowly added to a solution of diethylenetriamine (0.588 mL, 5.862 mmol) and 2 mL TEA in 10 mL CH_2Cl_2 at 0 °C. The reaction mixture was stirred for 2 h, and the slurry was diluted with additional CH_2Cl_2 and washed with 1 M NaOH. The organic layer was dried with Na_2SO_4, and the solvent was concentrated under reduced pressure. The residue was purified by silica gel column chromatography with CH_2Cl_2/methanol (5:1, v/v) with 1-2% TEA as the eluent to yield N-(2-((2-aminoethyl)amino)ethyl)-4-methoxybenzamide. Yield: 20%. ^{1}H NMR ($CDCl_3$): δ 7.79 (d, 2H), 6.90 (d, 2H), 3.82 (s, 3H), 3.91 (s, 24H), 3.27 (m, 2H), 2.90 (m, 4H), 2.75 (m, 2H). N-(2-((2-aminoethyl)amino)ethyl)-4-methoxybenzamide (130 mg, 0.55 mmol) was reacted with PEG_{2K}-bis-carboxylate (1.2 g, 0.55 mmol) in 80 mL anhydrous CH_2Cl_2 in the presence of N,N'-dicyclohexylcarbodiimide (230 mg, 1.1 mmol) and 4-dimethylaminopyridine (135 mg, 1.1 mmol) under N_2 for 48 h. After solvent concentration, 300 mL diethyl ether was added to the mixture, and the crude product precipitated out and was isolated by centrifugation. The product was purified by silica gel column chromatography with CH_2Cl_2/methanol (10:1 v/v) as the eluent to afford AA-PEG_{2K}-mono-carboxylate. Yield: 35%. ^{1}H NMR ($CDCl_3$): δ 7.77 (m, 2H), 6.89 (d, 2H), 4.20 (m, 2H), 3.95 (s, 3H), 3.81 (t, 4H), 3.61 (s, 180H) 3.44 (m, 2H). AA-PEG_{2K}-mono-carboxylate (300 mg, 0.13 mmol) was reacted with DSPE (115 mg, 0.15 mmol) in 25 mL anhydrous CH_2Cl_2 in the presence of N, N'-dicyclohexylcarbodiimide (53 mg, 0.26mmol) and 4-dimethylaminopyridine (31.3 mg, 0.26 mmol) under N_2 for 48 h. After solvent concentration, 50 mL diethyl ether was added to the mixture, and the crude product precipitated out and was isolated by centrifugation. The product was then purified by silica gel column chromatography with CH_2Cl_2/methanol (10:1 v/v) as the eluent to afford AA-PEG_{2K}-DSPE. Yield: 30%.^{1}H NMR ($CDCl_3$): δ 7.78 (m, 2H), 6.88 (d, 2H), 4.22 (m, 4H), 3.81 (s, 3H), 3.62 (s, 180H), 2.25 (s, 6H), 1.57 (s, 10H), 1.23 (S, 30H), 0.85 (t, 6H).

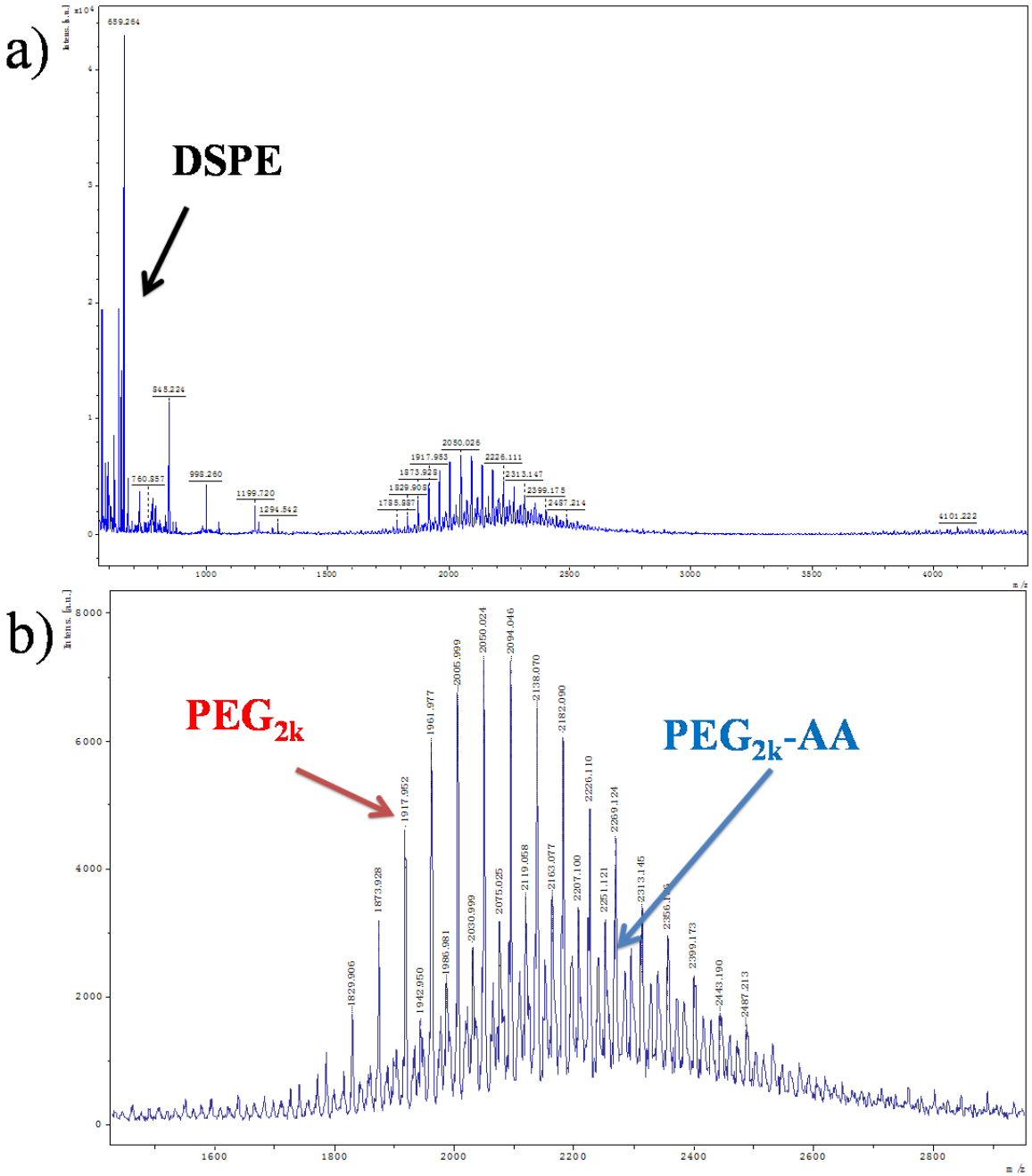

Figure S1. (a) MALDI-TOF mass spectrum of DSPE-PEG-AA at a range of 700-4500 Da and (b) 1450-2950 Da. The DSPE peak can be observed at 659 Da. In the region of 1800-2500 Da, two broad, distinctive peaks were observed. The peak between 1800-2450 Da with the maximum intensity at 2050 Da results from the PEG_{2k} fragment. The other smaller peak between 1850-2500 Da with the maximum intensity at 2182 Da is due to the PEG_{2k}-AA fragment. Each of the board peak contained sharp peaks separated by m/z=44, which corresponds to one subunit of PEG. The DSPE group is fragmented during the MALDI analysis, which was supported by the observed PEG peak in the MALDI-TOF spectrum of DSPE-PEG under similar conditions.

3. Determination of Drug Loading and Release Profile.

1) **Drug loadings of 1.** By measuring the absorbances of zoledronic acid in five different concentrations in 0.1 M HCl at 222 nm, corresponding standard curve was made. Particles were digested in 0.1 M HCl overnight. The concentration of drug in the solution was determined by the absorbance at 222 nm recorded. Drug loading of **1** bare particle is 63±5 wt. %, drug loading after lipid coating and pegylation is 42±7 wt%, drug loading after anisamide targeting is 40±4 wt%.

2) **Release profiles of 1 and 1@peg.** By measuring the absorbances of zoledronic acid in five different concentrations in 5 mM PBS at maximum absorption wavelengths, corresponding standard curve was made. Drug released from dialysis tubing with 3500 MW cutoff was monitored by UV-Vis in 5 mM PBS at 37 ^{o}C.

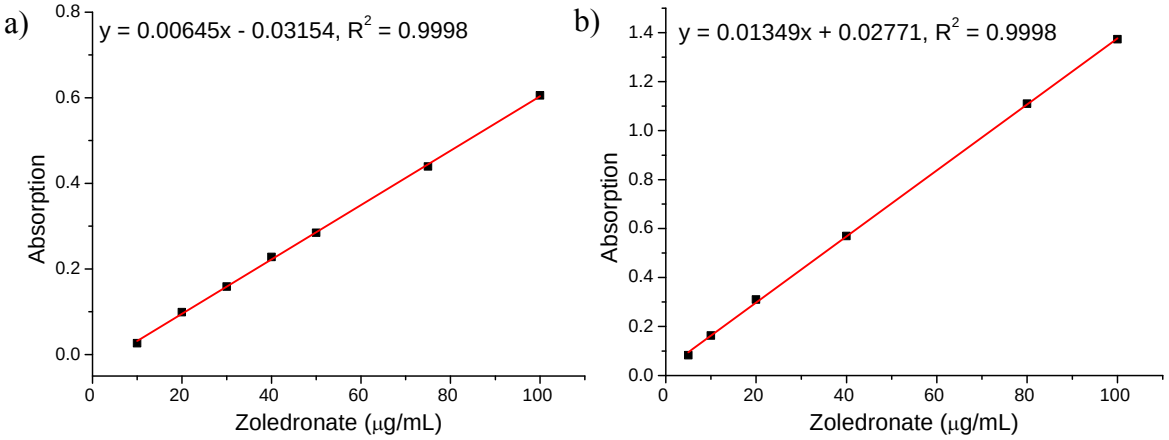

Figure S2. (a) Calibration curve of UV-Vis absorption of zoledronic acid in 0.1 M HCl. (b) Calibration curve of UV-Vis absorption of zoledronic acid in 5 mM PBS buffer.

4. Determination of Mn uptake by MCF-7 cells.

Confluent MCF-7 cells were trypsinized and counted on a hematocytometer. Culture flasks were seeded with 1 x 10^6 cells/flask in 10 mL media. Flasks were incubated at 37°C and 5% CO_2 overnight for cells to adhere. Media was removed from the flasks and each flask was washed with phosphate buffered saline (PBS). Each flask received 10 mL media and 0.25mg nanoparticles. The control flask was prepared as the other flasks but without particles. The flasks were incubated for 8h and 24 h. Media was removed, and each flask was washed twice with 5 mL PBS before being trypsinized. The cells were isolated by centrifuging and washed with PBS and centrifuged again. Cell pellets were digested in concentrated HNO_3 and Mn content was analyzed by ICP-MS.

5. *In vitro* cytotoxicity assay.

1) **Cell Lines.** MCF-7 human breast cancer cells (ATCC# HTB-22) and AsPC-1 (ATCC# **CRL-1682)** human pancreatic adenocarcinoma cancer cells were purchased from the Tissue Culture Facility of the Lineberger Comprehensive Cancer Center at the University of North Carolina at Chapel Hill. MCF-7 cells were cultured in DMEM growth medium and AsPC-1 cells were cultured in RPMI-1640 growth

medium (Cellgro), both were supplemented with 10% fetal bovine serum (Sigma) and 2% penicillin-streptomycin (Sigma).

2) **Cytotoxicity assay against MCF-7 cells.** Confluent MCF-7 cells were trypsinized (trypsin-EDTA, Sigma) and cell density was obtained from a hemocytometer. 6-well plates were seeded with $5.0x10^4$ cells/well and a total of 3 mL media. The plates were incubated at 37°C and 5% CO_2 overnight. Amounts of zoledronate, liposomes, and particle dispersions in RPMI-1640 medium and additional media (5% phosphate buffered saline, Cellgro) were added to wells resulting in zoledronate concentrations of 0, 2, 4, 6, 8, and 15 μM. The plates were incubated at 37°C and 5% CO_2 for 48 h and viability was determined via the trypan blue exclusion assay.

3) **Cytotoxicity assay against AsPC-1 cells.** Confluent AsPC-1 cells were trypsinized (trypsin-EDTA, Sigma) and cell density was obtained from a hemocytometer. 6-well plates were seeded with $5.0x10^4$ cells/well and a total of 3 mL media. The plates were incubated at 37°C and 5% CO_2 overnight. Amounts of zoledronate, liposomes, and particle dispersions in RPMI-1640 medium and additional media (5% phosphate buffered saline, Cellgro) were added to wells resulting in zoledronate concentrations of 0, 2.5, 5, 10, 20, and 40 μM. The plates were incubated at 37°C and 5% CO_2 for 48 h and viability was determined via the trypan blue exclusion assay.

6. Chlorin e6 doped 1(1').

A w=7.4 microemulsion was prepared by the addition of 0.2 mL of a 25mg/mL ZoL-Na_4 aqueous solution, 20uL of chlorin e6 sodium salt solution and 0.22 mL of a 100mg/mL $MnCl_2$ aqueous solution to separate 5.5 mL aliquots of a 0.3 M Triton X-100/1.5 M 1-hexanol in cyclohexane mixture while vigorously stirring at room temperature. 20 uL DOPA (200mg/mL in $CHCl_3$) was added to the complex solution and the stirring was continued for 15mins until clear solution formed. The two microemulsions were combined, and the resultant 11 mL microemulsion with w=7.4 was stirred for an additional 30 minutes. After the adding of 20 mL ethanol, **1'** were washed once with ethanol and twice with 50% EtOH/THF, and redispersed in THF.

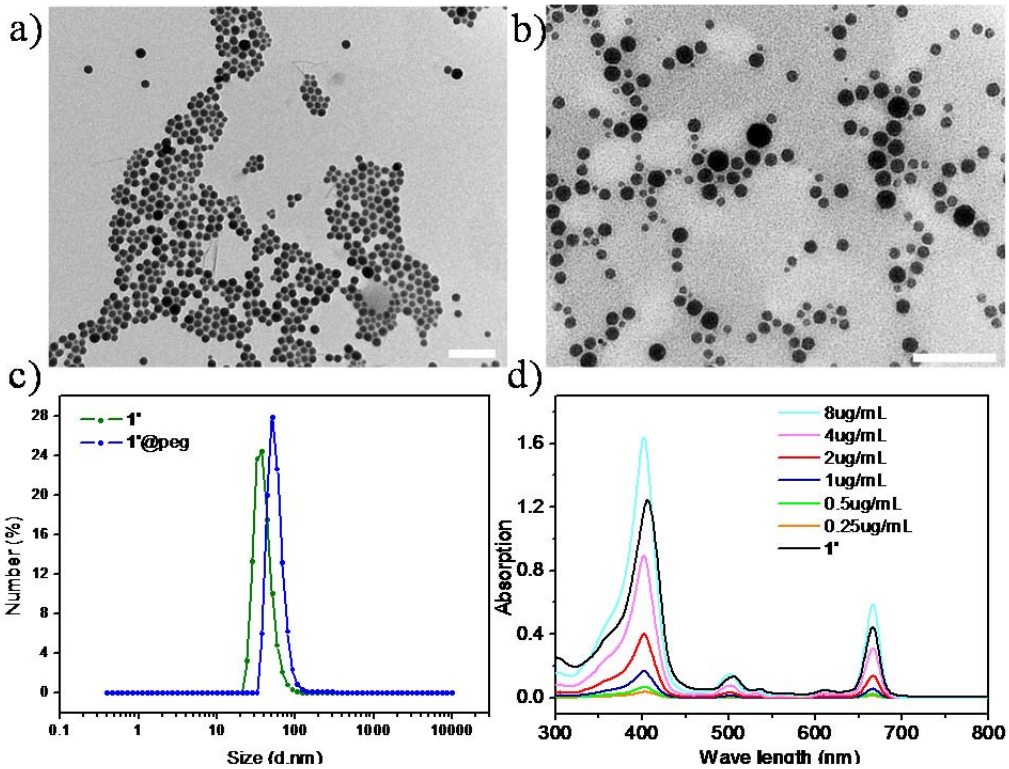

Figure S3. TEM images of **1'** (a) and **1'**@peg (b). c) Size distribution of **1'** and **1'**@peg measured by DLS. d) UV-Vis absorption spectra of chlorin e6 and **1'** in THF. Scale bars = 200 nm.

7. **Visualization of the NCP internalization by confocal laser scanning microscopy.** To visualize the internalization of **1**@peg in endosomal/lysosomal compartments, MCF cells were incubated with **1'**@peg and **1'**@peg-AA for 4 h at 37 °C. The cells were washed with PBS three times, fixed with 4% paraformaldehyde, and stained with Lysotracker Green (100 nM) before observation via confocal laser scanning microscopy (CLSM, Olympus FV1000). Co-localization of the Ce6 fluorescence (red) and Lysotracker Green indicates the uptake of NCPs via endocytosis pathways.

8. **Estimation of relaxivity *in vitro***.
The *in vitro* longitudinal relaxivity was estimated by a combination of *in vitro* MRI study and ICP-MS uptake study. Assuming the density of cell to be 1 g/cm3, the concentration of Mn inside cell was determined to be 0.67 mM and 2.70 mM for **1**@peg and **1**@peg-AA by ICP-MS. T_1^{-1} values were obtained from MRI measurements to be 9.3 s^{-1} and 25.4 s^{-1}. The slope of these two data points was used to establish the r_1 value to be ~8.0 mM^{-1} s^{-1}.