Supporting Information

Second Generation Nanosponges: Nanonetworks in Controlled Dimensions via Backbone Ketoxime and Alkoxyamine Cross-Links for Controlled Release

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Materials. SiliaMetS® Cysteine was purchased from Silicycle. Spectra/Por® dialysis tubing (1kD MWCO) and Float-a-Lyzer® dialysis devices (1000kD MWCO) were purchased from Spectrum Labs. SnakeSkin™ dialysis tubing (10kD MWCO) was purchased from ThermoFisher Scientific. O,O'-(((oxybis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl))bis(hydroxylamine), also referred to as bis(aminooxy)-PEG3, was purchased from Broadpharm. 1,4-cyclohexanedione was purchased from Tokyo Chemical Industry America. All other reagents and solvents were purchased from Sigma-Aldrich and used without further purification unless otherwise noted. δ-Valerolactone was purified via vacuum distillation prior to polymerization.

Characterization. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AV-I 400, JEOL ECX-400, and JEOL ECA-600 II spectrometers. Chemical shifts were referenced to solvent resonance signals. Gel permeation chromatography (GPC) was conducted on a ToSOH EcoSEC HLC-8320GPC system equipped with a refractive index detector, UV-8320 detector, and TSKgel H_{HR} columns (7.8x300mm G5000H_{HR}, G4000H_{HR}, and G3000H_{HR}) with tetrahydrofuran (THF) as the eluent at a flow rate of 1 mL/min. Transmission Electron Microscopy (TEM) was performed using an JEOL 2000-FX microscope operated at 200kV. Samples for transmission electron microscopy (TEM) were prepared by dissolving nanoparticles (~0.5 mg) in 0.22 μm filtered acetonitrile (ACN). The samples were then stained with 3 drops of 3% phosphotungstic acid monohydrate and vortexed. Carbon grids were prepared by dipping an Ultrathin Carbon Type-A 400 Mesh Copper Grid in the sample solution three times and allowing to dry at ambient temperature for 12 h. Dynamic light scattering (DLS) was performed on a Malvern Zetasizer Nano system with a fixed angle of 173˚ at 25˚C. All particles were measured in 0.22 μm filtered THF diluted to a concentration that produced the desired count rate with a low signal-to-noise

ratio. Static light scattering was performed using the Molecular Weight function of a Malvern Zetasizer Nano system in THF between 0.01-0.1 mg/mL concentrations. High performance liquid chromatography (HPLC) analysis of drug concentration was conducted using a ThermoFisher Ultimate 3000 HPLC system and Phenomenex column (Luna 5µ C8(2) 100Å, 150 x 4.6 mm, 5µm) with an isocratic mixture of methanol and water (57:43) and flow rate of 1.0 mL/min at 230 nm.

Synthesis of 2-oxepane-1,5-dione (OPD). Procedure modified from literature.[1] Meta-chloroperoxybenzoic acid (11.99 g, 54 mmol) and 1,4-cyclohexanedione (4.0 g, 36 mmol) was dissolved in anhydrous dichloromethane (DCM) (45.5 mL) and refluxed for 3 h. The reaction mixture was cooled to room temperature and a white precipitate was removed by gravity filtration. The precipitate was washed with excess DCM (10 mL) to solubilize any residual product. The organic layer was dried over anhydrous magnesium sulfate and dried in vacuo to collect a white solid. Diethyl ether (10 mL) was added to the flask to wash and collect a white solid via vacuum filtration (80% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.70-2.73 (m, 2H); 2.81-2.85 (m, 4H); 4.42 (t, 2H) ^{13}C NMR (600 MHz, $CDCl_3$): δ 27.8, 38.5, 44.6, 63.3, 173.5, 205.1

General synthesis of poly(δ-valerolactone-2-oxepane-1,5-dione) (P(VL-*co*-OPD)). Tin(II) trifluoromethanesulfonate (3.93 mg, 9.25×10^{-3} mmol), isoamyl alcohol (81.72 µL, , 7.5×10^{-1} mmol), and DCM (2.61 mL) were added to a flame dried and N_2 purged flask. δ-valerolactone (VL, 2.57 mL, 27 mmol) and 2-oxepane-1,5-dione (OPD, 0.22 g, 1.8 mmol) were added at 0 °C then stirred at room temperature for 18 h. The reaction mixture was quenched with excess methanol (3 mL) and a solid-support metal scavenger (SiliaMetS® Cysteine, 150 mg) was added and stirred for 2 h to capture tin catalyst. The mixture was filtered via gravity filtration and

transferred to dialysis tubing (1kD MWCO) and dialyzed against methanol (MeOH)/DCM (1:1) mixture for 24 h, with frequent solvent changes. After dialysis, the solvent was removed under reduced pressure and product dried in vacuo to obtain a light yellow, waxy product. (56% yield) ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (d, 6H); 1.6-1.75 (m); 2.23-2.43 (m); 2.25-2.7 (m); 2.7-2.84 (m); 3.4 (t); 3.65 (m); 4.0 (t); 4.3-4.4 (m). ^{13}C NMR (600 MHz, $CDCl_3$): δ 21.25, 27.9, 33.5, 63.8, 173.2, 205.75

General synthesis of ketoxime nanoparticle (NP_{KO}). P(VL-*co*-OPD) (4% OPD, 50 mg, 4556.20 g mol^{-1}, 2925.14 g mol^{-1} keto-group, 1.71×10^{-5} mol) was dissolved in dichloromethane (5.70 mL) then added to a 50 mL round bottom flask. O,O'-(((oxybis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl))bis(hydroxylamine) (3.83 mg, 1.71×10^{-5} mol, 1 equiv.) was dissolved in dichloromethane (0.63 mL) and added quickly to the polymer solution at a fast vortex. The reaction was stirred for 2 h then immediately transferred to Thermo Scientific™ SnakeSkin™ 10K MWCO Dialysis Tubing. The solution was dialyzed against dichloromethane for 48 h, changing the solvent 3-4 times per day. The solvent was removed via rotary evaporation. The product was dried in vacuo to yield a light tan waxy solid (80% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (d, 6H); 1.6-1.75 (m); 2.23-2.43 (m); 2.25-2.7 (m); 2.7-2.84 (m); 3.4 (t); 3.65 (m); 4.0 (t); 4.3-4.4 (m). ^{13}C NMR (600 MHz, $CDCl_3$): δ 21.32, 25.53, 28.00, 33.62, 63.87, 67.98, 173.30

General synthesis of alkoxyamine nanoparticle (NP_{AA}). To ketoxime nanoparticles (50 mg) formed *in situ*, sodium cyanoborohydride (2.15 mg, $3.42\text{x}10^{-5}$ mol, 2 equiv.) and a catalytic amount of saturated sodium bicarbonate solution (100 µL) were added directly to the reaction flask. The reaction stirred for 2 h then was transferred to Thermo Scientific™ SnakeSkin™ 10K

MWCO Dialysis Tubing. The solution was dialyzed against a 1:1 mixture of MeOH/DCM for 48 h, with 3-4 solvent changes per day. The solution was filtered with a 0.45 μm filter to remove solid salt particulates and solvent was removed via rotary evaporation. The product was dried in vacuo to yield a light tan waxy solid (80% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (d, 6H); 1.6-1.75 (m); 2.23-2.43 (m); 2.25-2.7 (m); 2.7-2.84 (m); 3.4 (t); 3.65 (m); 4.0 (t); 4.3-4.4 (m). ^{13}C NMR (600 MHz, $CDCl_3$): δ 21.32, 25.53, 28.00, 33.62, 63.85, 67.88, 173.26

General synthesis of partially reduced ketoxime/alkoxyamine nanoparticle ($NP_{KO/AA}$). To ketoxime nanoparticles (348 mg) formed *in situ*, sodium cyanoborohydride (6.72 mg, $1.07x10^{-4}$ mol, 0.5 equiv.) and a catalytic amount of saturated sodium bicarbonate solution (100 μL) were added directly to the reaction flask. The reaction stirred for 2 h then was transferred to Thermo Scientific™ SnakeSkin™ 10K MWCO Dialysis Tubing. The solution was dialyzed against a 1:1 mixture of MeOH/DCM for 48 h, with 3-4 solvent changes per day. The solution was filtered with a 0.45 μm filter to remove solid salt particulates and solvent was removed via rotary evaporation. The product was dried in vacuo to yield a light tan waxy solid (80% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (d, 6H); 1.6-1.75 (m); 2.23-2.43 (m); 2.25-2.7 (m); 2.7-2.84 (m); 3.4 (t); 3.65 (m); 4.0 (t); 4.3-4.4 (m). ^{13}C NMR (600 MHz, $CDCl_3$): δ 21.32, 25.53, 28.00, 33.62, 63.85, 67.88, 173.26

In vitro nanoparticle degradation studies. NPs (14% OPD, 5.4 mM, ~170 nm) were suspended in 2 mL of 0.1M acetic acid-NaOAc buffer with 0.1% v/v Tween® 80 (pH 5.0) or phosphate buffered saline with 0.1% v/v Tween® 80 (PBS, pH 7.4) in 1 dram vials with a micro stir bar. The vials were sealed and samples were continuously stirred at 37˚C. At 48 h intervals, nanoparticles

and degradation products were extracted with dichloromethane (3 x 3 mL). The organic layer was dried over magnesium sulfate and dried *in vacuo*. The degradation of particles was monitored via static light scattering utilizing the Molecular Weight function of a Malvern Zetasizer Nano instrument.

General nanoprecipitation procedure for encapsulation of Brefeldin A into nanoparticles. NPs (12.6 mg, 8% OPD, 2.7 mM, ~80 nm) were added to a 1.5 mL centrifuge tube. Brefeldin A (BFA) was solubilized in dimethyl sulfoxide (DMSO) to a known concentration and then added to the NPs (3.15 mg, 11.2 mmol). Additional DMSO was added to the mixture up to a total of 50 µL. Cell culture grade water containing 0.1% D-α-tocopherol polyethylene glycol 1000 succinate (1 mL) was added to the centrifuge tube and vortexed to induce BFA encapsulation. D-α-tocopherol polyethylene glycol 1000 succinate is added to coat the particles during encapsulation and aid in resuspension in aqueous media during drug release studies. The mixture was then centrifuged at 14000 RPM for 20 min. The supernatant was decanted, fresh cell culture grade water (1 mL) was added to the particle pellet and vortexed until particles were resuspended. Centrifugation was repeated at 14000 RPM for 20 min, then the supernatant was decanted to remove any unincorporated drug. Cell culture grade water (0.5 mL) was added to the mixture, frozen, and lyophilized to produce BFA encapsulated nanoparticles (BFA-NP). HPLC analysis confirmed encapsulation of BFA at an average of 19.9 wt % with 99.7% efficiency.

***In vitro* release of Brefeldin A from nanoparticles.** BFA-NPs (~8 mg, ~20 wt% BFA) were weighed into a 1.5 mL centrifuge tube and 1 mL 0.1M acetic acid-NaOAc buffer with 0.1% v/v Tween® 80 (pH 5.0) or phosphate buffered saline with 0.1% v/v Tween® 80 (PBS, pH 7.4) was

added. Tween® 80 is added to the media to promote BFA suspension in the media after release from particles and reduce drug-vessel interactions. The mixture was vortexed until the particles were suspended in the media, and then the particles were transferred to a 1000kD Float-a-Lyzer™ dialysis membrane. The dialysis device was placed in a 50 mL centrifuge tube containing 18 mL of dialysis media and a small stir bar. The centrifuge tube was submerged in a water bath at 37℃ and stirred, and aliquots (150 µL) were removed from the dialysis media at specified time points and replaced with fresh media. BFA concentration in the aliquots was determined via HPLC at 230 nm with isocratic gradient of 57% MeOH : 43% water. Flow rate of 1 mL/min with run time of 10 min yielded a retention time of 7.5 min.

Control experiments for particle-drug interaction of Brefeldin A and ketoxime nanoparticles. Brefeldin A (0.2 mg, 0.71 mmol) was added to a 1 dram vial containing either O,O'-(((oxybis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl))bis(hydroxylamine) (0.1 mg, 0.45 mmol) or P(VL-*co*-OPD) (8% OPD, 0.7 mg, 0.16 mmol) and mixed with minimal DMSO (20 µL). The mixture was diluted in 0.1M acetic acid-NaOAc buffer with 0.1% v/v Tween® 80 (pH 5.0, 1.5 mL) and stirred at 37℃ for 24 h. The resulting mixture was analyzed at 0 and 72 h post reaction via HPLC at 230 nm to evaluate any particle-drug interactions (**Figure S7**).

Table S1. GPC and NMR analysis of P(VL-*co*-OPD).

Monomer Feed Ratio (VL:OPD)	% OPD_{th}	% OPD[a]	OPD conv (%)	Polymer Composition (VL:OPD)	$M_{n\ theo}$ (g/mol)	M_n[a] (g/mol)	M_n[b] (g/mol)	M_w[b] (g/mol)	M_w/M_n[b] (g/mol)
94:6	6	3.76	63	96:4	4000	4588	3000	4000	1.31
85:15	15	7.52	50	92:8	4000	4420	2800	3900	1.39
70:30	30	14.18	47	86:14	4000	3637	2800	3600	1.31

[a] % OPD and M_n determined by 400 MHz ^{1}H NMR in $CDCl_3$. [b] Molecular weight and polydispersity measured by GPC at 40℃ in THF and a flow rate of 1 mL/min using using ToSOH EcoSEC HLC-8320GPC system equipped with a refractive index detector, UV-8320 detector, and TSKgel H_{HR} columns (7.8x300mm G5000H_{HR}, G4000H_{HR}, and G3000H_{HR}).

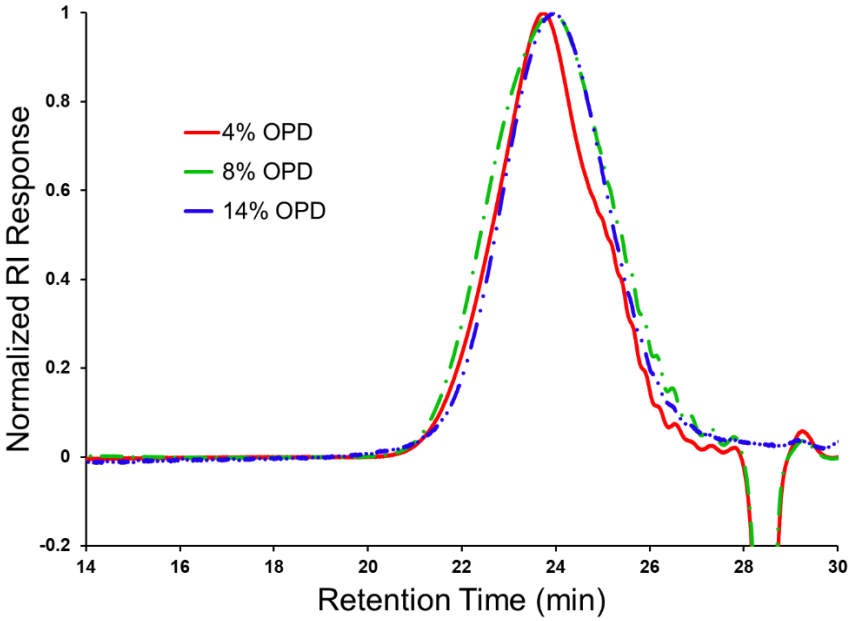

Figure S1. GPC traces of P(VL-*co*-OPD) at varying comonomer ratios, measured in THF at 40℃ with a flow rate of 1 mL/min.

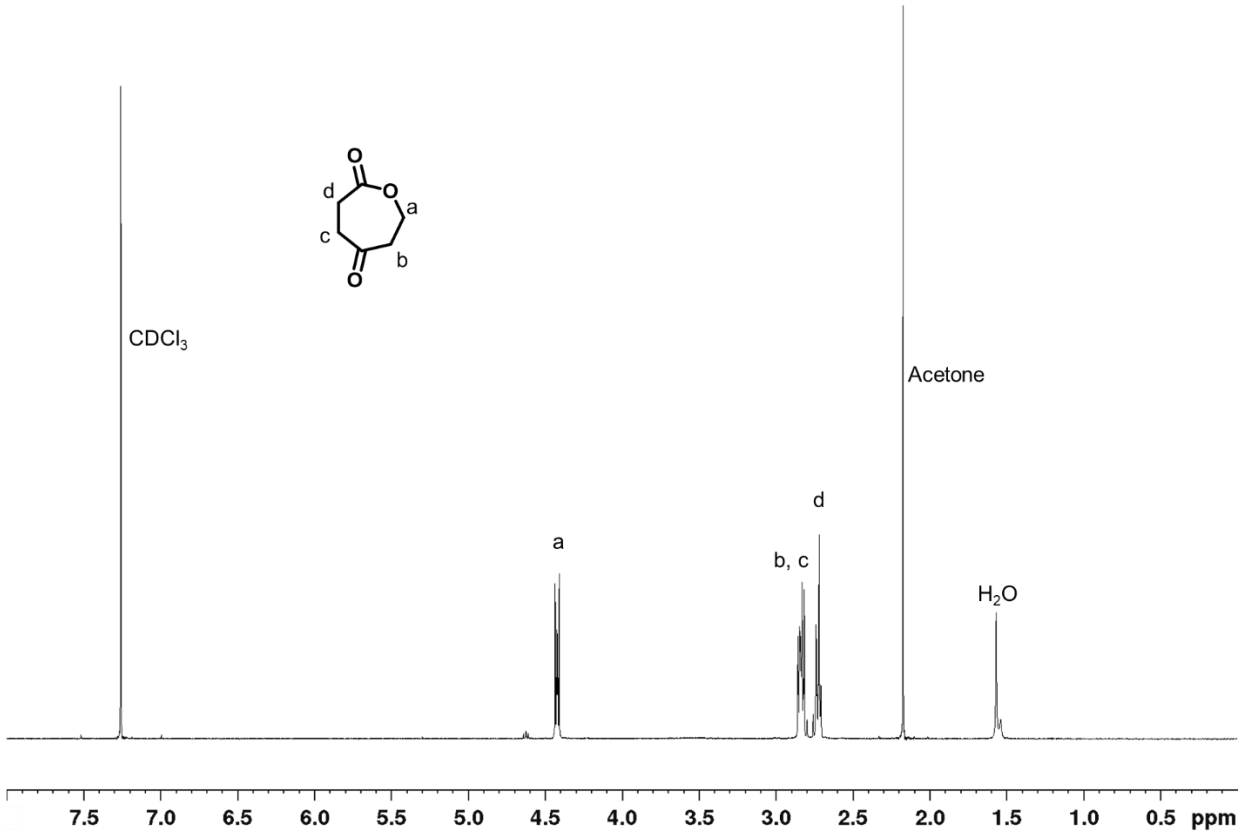

Figure S2. 400 MHz ^{1}H NMR spectra of 2-oxepane-1,5-dione in $CDCl_3$.

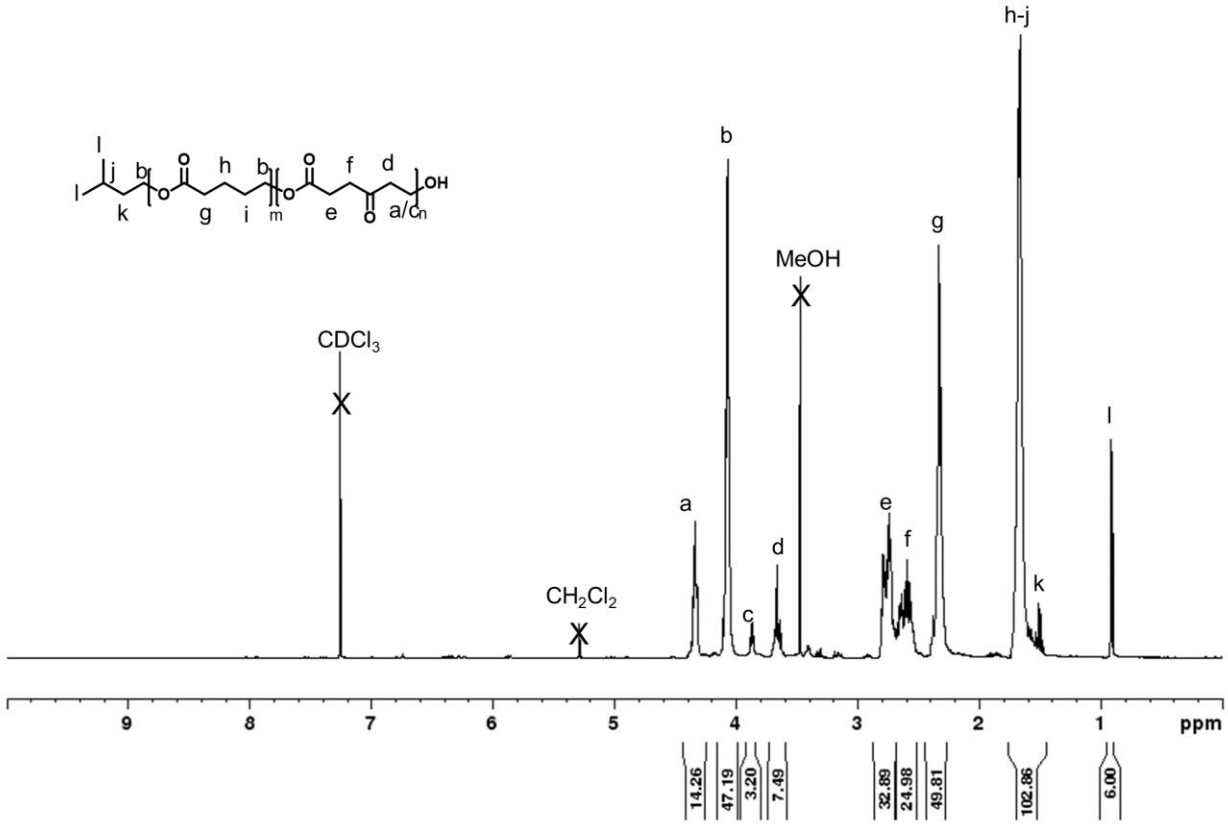

Figure S3. 400 MHz ^{1}H NMR spectra of VL-*co*-OPD in $CDCl_3$.

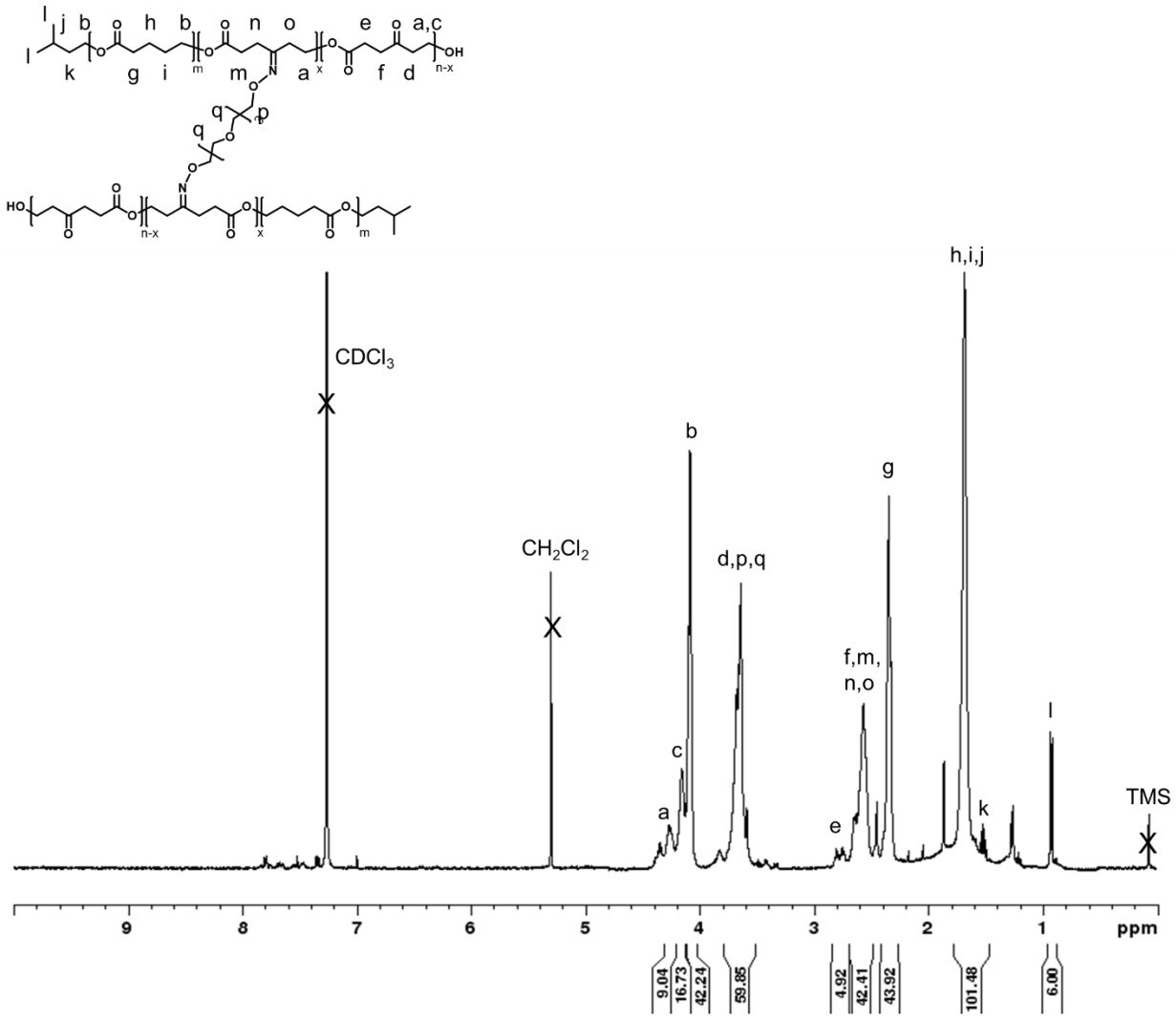

Figure S4. 400 MHz ^{1}H NMR characterization of ketoxime nanoparticles in $CDCl_3$.

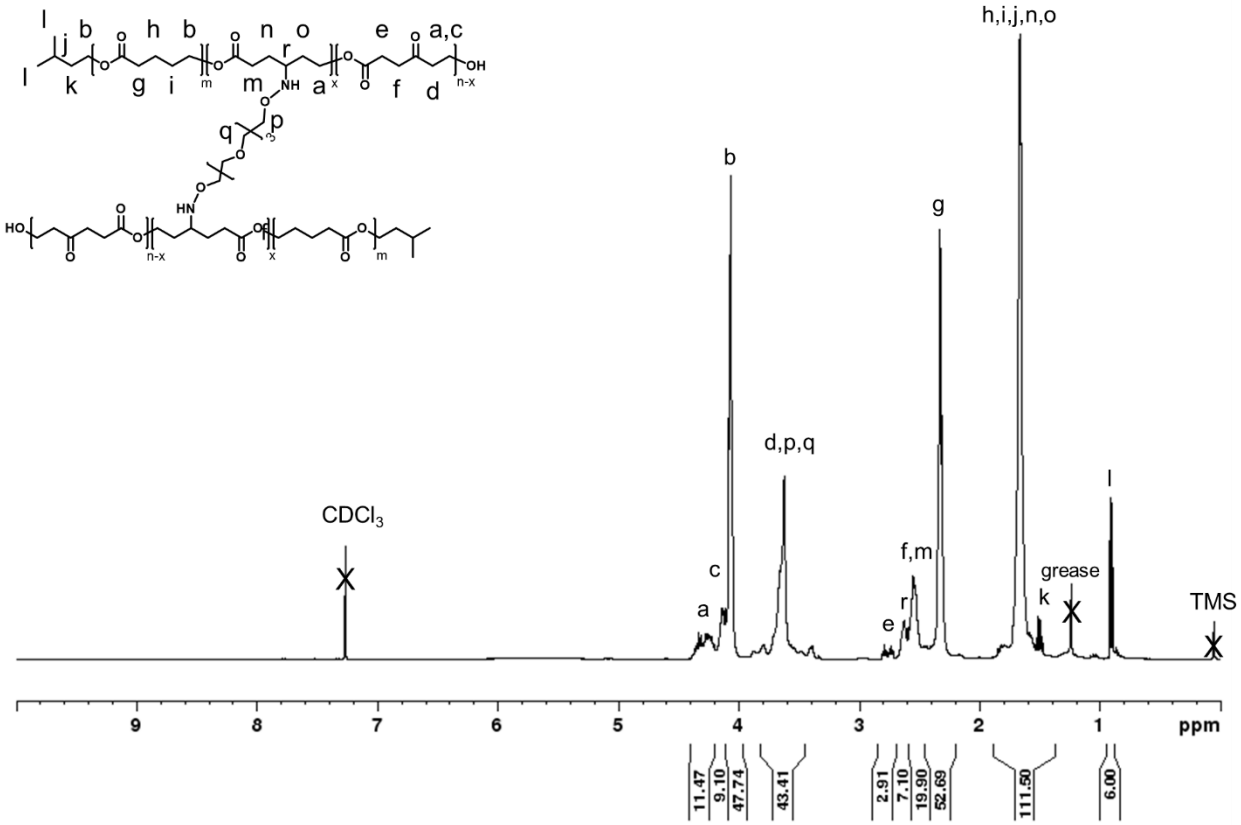

Figure S5. 400 MHz ^{1}H NMR characterization of alkoxyamine nanoparticles in $CDCl_3$.

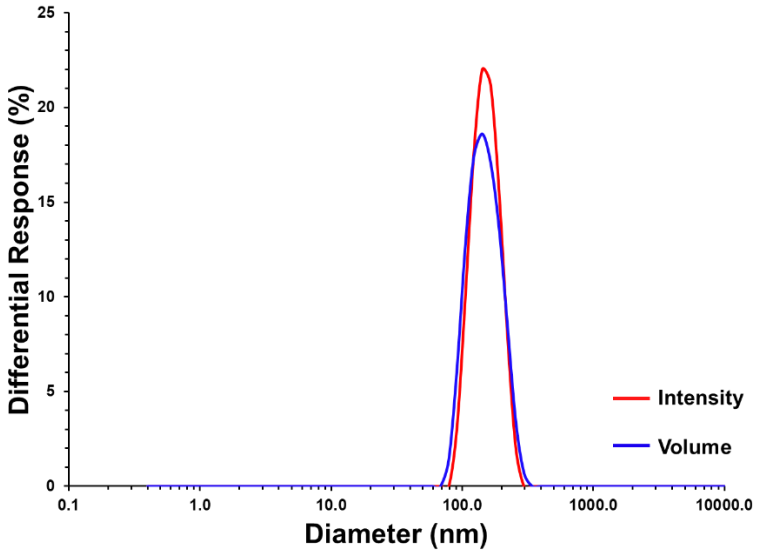

Figure S6. Representative dynamic light scattering data of particles measuring ~165 nm.

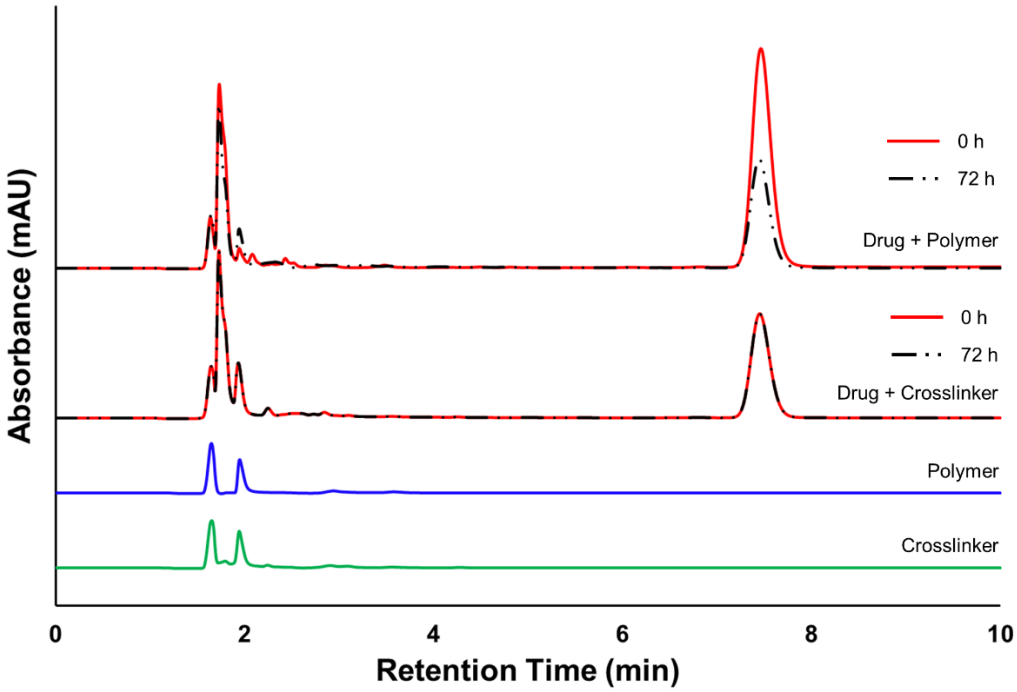

Figure S7. HPLC analysis of control experiment with physical mixture of drug (BFA) and polymer (P(VL-*co*-OPD)) or crosslinker (bis(aminooxy) PEG-3) in pH 5.0 at 0 and 72 h post reaction. Reduced absorbance of BFA (ret. time = 7.8 min) of drug + polymer mixture after 72 h indicates presence of particle-drug interaction.

REFERENCES

1. Jean-Pierre Latere, P. L., Philippe Dubois, Robert Jerome, 2-Oxepane-1,5-dione: A Precursor of a Novel Class of Versatile Semicrystalline Biodegradable (Co)polyesters. *Macromolecules* **2002,** *35* (21), 7857-7859.