

Supporting Information

Composite Nanomaterials by Self-assembly and Controlled Crystallization of Poly(2-isopropyl-2-oxazoline)-Grafted Polysaccharide

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Methods

Synthesis of 2-isopropyl-2-oxazoline. Isobutyric acid (132 g, 1.5 mol) was added to 2-aminoethanol (92 g, 1.5 mol) under stirring. The resulting salt was heated slowly to 230 °C, a temperature higher than its melting point. The liquid was refluxed for 48 h, followed by azeotropic distillation at 110 °C. The distillate was diluted in dichloromethane (350 mL) to separate water from 2-isopropyl-2-oxazoline. The aqueous layer was extracted repeatedly with dichloromethane. The combined organic layers were concentrated in vacuo. The residue was purified by repeated distillations in the presence of CaH₂, yielding dry 2-isopropyl-2-oxazoline (72 g, b.p. 138°C). ¹H NMR (CDCl₃, δ): 1.11 (d, 6 H, (CH₃)₂CH-), 2.49 (m, 1H, (CH₃)₂CH-), 3.79 (t, 2 H, NCH₂CH₂O), 4.18 ppm (t, 2H, NCH₂CH₂O).

Preparation of Hydroxy-terminated Poly(2-isopropyl-2-oxazoline) (PIPOZ). A solution of 2-isopropyl-2-oxazoline (4.91 g, 43.4 mmol) and methyl *p*-tosylate (0.18 g, 0.98 mmol or 0.46 g, 2.46 mmol) in acetonitrile (20 mL) was stirred at 80 °C under argon. After 48 h of polymerization, the polymerization solutions were cooled to room temperature and added a mixture of aqueous NaOH (15 mL, 0.1 M) and methanol (15 mL). The resulting solution was dialyzed against water for 3 days and isolated by freeze-drying. ¹H NMR (CDCl₃, δ): 1.1 (br s, [(CH₃)₂CH-], 2.66 and 2.89 (br m, [(CH₃)₂CH-], 3.08 (s, terminal CH₃), 3.54 (br s, -(CH₂-CH₂-N)-).

Synthesis of Activated PIPOZ. To couple the PIPOZ to pullulan, the hydroxyl group of the PIPOZ was activated using *N,N'*-carbonyldiimidazole (CDI). CDI (48.6 mg, 300 μmol, 3.75 equiv) was dissolved in dried methylene chloride (5 mL) in a nitrogen atmosphere. PIPOZ (e.g., PIPOZ7K: 600 mg, 81.0 μmol, 1 equiv) was dissolved in methylene chloride (5 mL) and added to the CDI solution. The reaction mixture was stirred for 4 h at room temperature in a nitrogen atmosphere. Thereafter, added with water (100 mL), to decompose the excess CDI and extracted twice. The organic layers were combined and dried over magnesium sulfate. After filtration, the organic solvent was removed under reduced pressure to yield the PIPOZ-Cl.

Size Exclusion Chromatography (SEC) -Multiangle Laser Scattering (MALLS) Measurements. Molecular weights and molecular weight distributions of PIPOZ were determined by SEC. SEC was performed on a SEC-MALLS system consisting of an Agilent 1100 isocratic pump, a set of TSK-gel α-M (particle size 13 μ, exclusion limit 1×10⁷ Da for polystyrene

in DMF) and a TSK-gel α -3000 (particle size 7 μ , exclusion limit 1×10^5 Da for polystyrene in DMF) (Tosoh Biosep) columns, a Dawn EOS multi-angle laser light scattering detector $\lambda = 690$ nm (Wyatt Technology Co.) and an Optilab DSP interferometric refractometer $\lambda = 690$ nm (Wyatt Technology Co.) under the following conditions: injection volume, 100 μ L; flow rate, 0.5 mL/min; eluent, DMF; temperature, 40 $^{\circ}$ C using dn/dc values of 0.084 mL/g (λ ; 690 nm, 40 $^{\circ}$ C, DMF). The degree of polymerization (DP) was calculated from the molecular weight (Mn) of PIPOZ divided by molecular weight of the monomer unit, 2-isopropyl-2-oxazoline.

Synthesis of PIPOZ grafted Pullulan. PIPOZ-grafted-Pullulan (PIPOZ-Pul) was synthesized using the same procedure as reported by de Jong *et al.*[1] Pullulan (Mn = 6.9×10^4 g/mol, Mw/Mn = 1.53) (146 mg) and DMAP (55.2 mg, 0.45 mmol, 0.5 equiv to glycopyranose units of pullulan) were dissolved in dried DMSO (10 mL). Next, PIPOZ7K-Cl (226 mg, 30.5 μ mol) dissolved in dry DMSO (10 mL) was added. The solution was stirred at room temperature for 4 days in a nitrogen atmosphere. The reaction mixture was dialyzed against water. The PIPOZ-Pul was collected by lyophilization. The DS was calculated by ^1H NMR of the CH_3 groups of the PIPOZ at 0.92-1.08 ppm, with the degree of polymerization and anomeric proton of pullulan at 5.26-5.41 ppm. ^1H NMR (D_2O): δ 0.92-1.08 (br s, $[(\text{CH}_3)_2\text{CH-}]$, 2.62-2.75 and 2.81-2.95 (br m, $[(\text{CH}_3)_2\text{CH-}]$, 3.31-4.06 (m, glucose unit 2H, 3H, 4H, 5H, and 6H), 4.85-4.97 (br, glucose unit 1H(1-6)), 5.26-5.41 (br, glucose unit 1H(1-4)).

[1] De Jong, S. J.; De Smedt, S. C.; Wahls, M. W. C.; Demeester, J.; Kettenesvan, B. J. J.; Hennink, W. E. *Macromolecules* **2000**, 33, 3680-3686.

Differential Scanning Calorimetry (DSC). DSC measurements were performed on a VP-DSC microcalorimeter (MicroCal Inc.) at an external pressure of ca. 180 kPa. The cell volume was 0.52 mL. The heating rate was 1.0 $^{\circ}\text{C min}^{-1}$. The instrument response time was set at 5.6 s. Data were corrected for instrument response time and analyzed using the software supplied by the manufacturer. Solutions for analysis had a polymer concentration of 3.0 mg/mL.

^1H NMR Measurements. ^1H NMR measurements (500 MHz, R-500, JEOL, Tokyo, Japan) were performed at various temperature from 25 $^{\circ}\text{C}$ to 70 $^{\circ}\text{C}$. Pul-PIPOZ solutions (3.0 mg/ mL) were dispersed in D_2O , and filtered through 0.45 μm filter.

Transmission Electron Microscopy (TEM) Observation. Specimens were prepared by dropping 10 μ L of heat-treated Pul-PIPOZ solution (10 mg/mL) on a grid and negatively staining with 0.5% uranyl acetate. TEM (H-600; Hitachi, Tokyo, Japan) observation was performed at an accelerating voltage of 100V.

X-ray Diffraction (XRD) Measurements. To obtain concentrated aqueous solution, heat-treated Pul-PIPOZ aqueous solution (10 mg/mL) was dried in vacuo, then re-dispersed in water (150 mg/mL). The solution was dropped and air-dried on Si plate. The XRD data for the specimens was collected in the 2θ range of 5° to 50° with CuK α radiation at 40 kV and 35 mA using a PW1710 diffractometer (Nippon Phillip, Tokyo, Japan).

Dynamic Light Scattering (DLS). DLS measurements were carried out with a Zetasizer Nano ZS instrument (Malvern Instruments, Malvern, U.K.) equipped with a PELTIER temperature control unit at a wavelength of 632.8 nm and a 173° detection angle. The hydrodynamic radius of PIPOZ-Pul was determined with a Laplace inversion program (CONTIN). PIPOZ-Pul solutions (3.0 mg/ mL) were dispersed in water, and filtered through 0.45 μ m filter.

Table S1 Characterization of PIPOZ

<i>Polymer</i>	<i>M_n</i> ^{a)}	<i>M_w/M_n</i>	<i>DP</i>	<i>T_{max}</i> ($^\circ$ C) ^{b)}	ΔH (kcal/mol) ^{b)}
PIPOZ3K-OH	3.2×10^3	1.16	29	52.5	0.61
PIPOZ7K-OH	7.4×10^3	1.08	65	48.8	1.22

a) Determined by SEC-MALS in DMF

b) Determined by high-sensitivity differential scanning calorimetry in H₂O

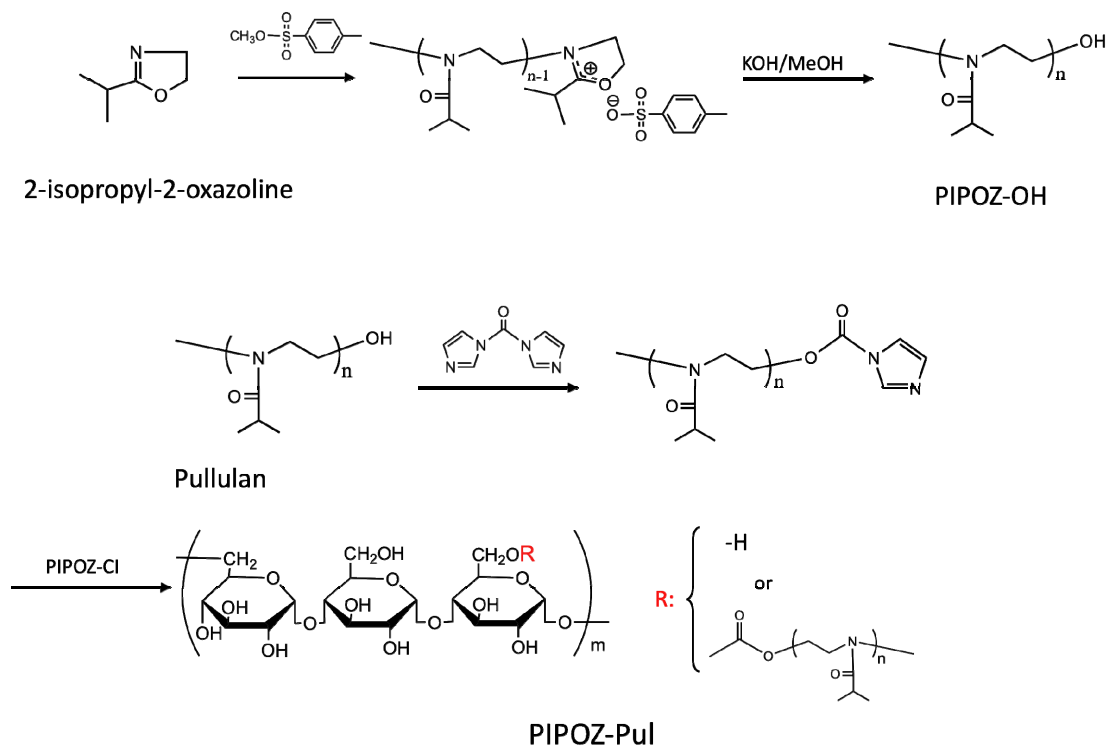


Figure S1 Synthetic route of PIPOZ-Pul

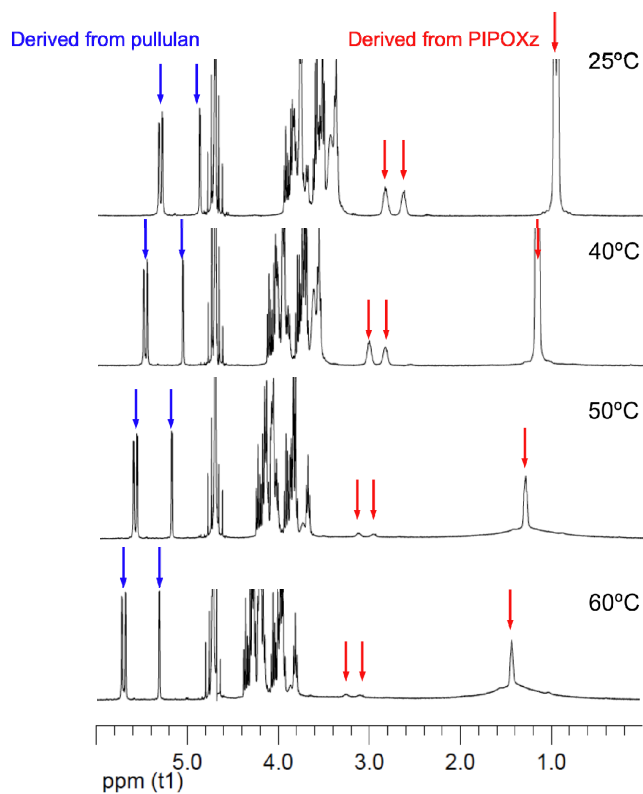


Figure S2 ^1H NMR spectra of PIPOZ7K-Pul at various temperature.

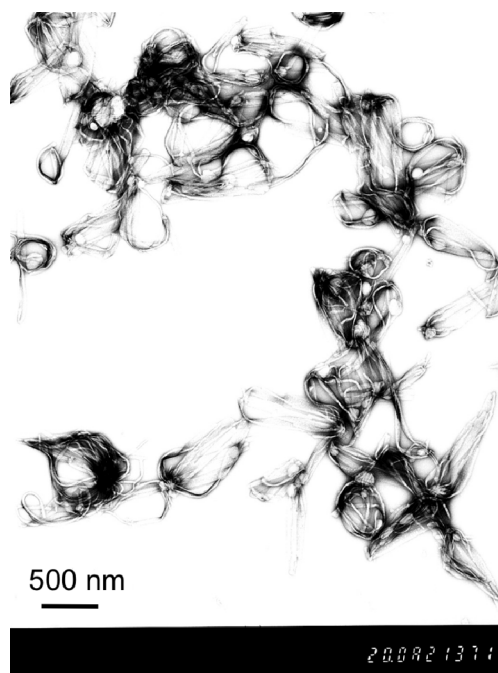


Figure S3 TEM images of insoluble fraction of heat treated (70 °C, 1h) PIPOZ7K (3.3 mg/mL) aqueous solutions.

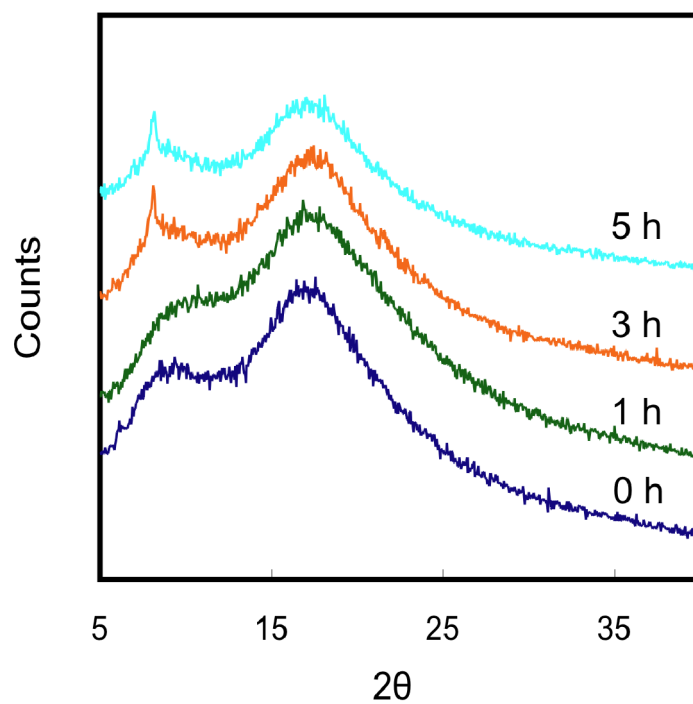


Figure S4 XRD spectrum of PIPOZ heated at 70 °C in water