Supporting Information

Green Synthesis of Methoxy-poly(ethylene glycol)-*block*-poly(L-lactide-*co*-glycolide) Copolymer Using Zinc Proline as a Biocompatible Initiator for Irinotecan Delivery to Colon Cancer *In Vivo*

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Supplementary methods

Characterization of the copolymer

^{1}H and ^{13}C NMR

Polymers P-1, P-2, and P-3 were dissolved in $CDCl_3$ at room temperature. ^{1}H- and ^{13}C-NMR spectra were recorded using 400 MHz Bruker 400 spectrometer using $CDCl_3$ as the solvent containing a small amount of the TMS as an internal standard.

ATR-FTIR

ATR-FTIR spectra of nanoparticles samples were recorded using a (Perkin Elmer IRFTIR spectrometer USA) in 500–4000 cm^{-2} wavelength range in an attenuated total reflectance (ATR) mode. The instrument was calibrated with an indium standard before measurements.

GPC

The molecular weights such as number-average molecular weight [Mn], weight-average molecular weight [Mw], and polydispersity [M_w/M_n] were determined with respect to polystyrene standards by size-exclusion chromatography on an Agilent Technologies, Polymer Laboratories Gel permeation chromatography (PL-GPC) 220 machine (Santa Clara, CA, USA) at 25 °C, with eluting m-PEG PLGA solutions (10 mg/mL of $CHCl_3$) and toluene as an internal standard, and through a series of five 30 cm long Styragel columns with pore sizes of 500, 10^5, 10^4, 10^3, and 100Å. $CHCl_3$ was used as the mobile phase (flow rate: 1 mL/min), and a refractive index detector was used for the detection of different molecular weight fractions.

MALDI-TOF MS

MALDI-TOF MS analysis was performed on an AB SCIEX4800 plus MALDI TOF/TOFTM Analyzer. The samples were dissolved in tetrahydrofuran (1mg mL^{-1}) and mixed with the matrix (15mg mL^{-1} of tetrahydrofuran) and dried on the sample plate. 2, 5-dihydroxybenzoic acid and dithranol were used as the matrix.

Transmission Electron Microscopy (TEM)

TEM imaging of nanoparticles was carried out using (FEI Technai G2 T20) instrument with an acceleration voltage of 200 keV. The TEM sample was prepared by transferring the nanoparticles suspension (4 mg mL^{-1}) onto a 200-mesh carbon-coated copper grid. Samples were blotted away after 30 min incubation and grids were negatively stained for 10 min at room temperature with freshly prepared, 2 % (w/v) phosphotungstic acid aqueous solution. The grids were then washed twice with distilled water and air-dried before imaging.

Supplementary figures/tables

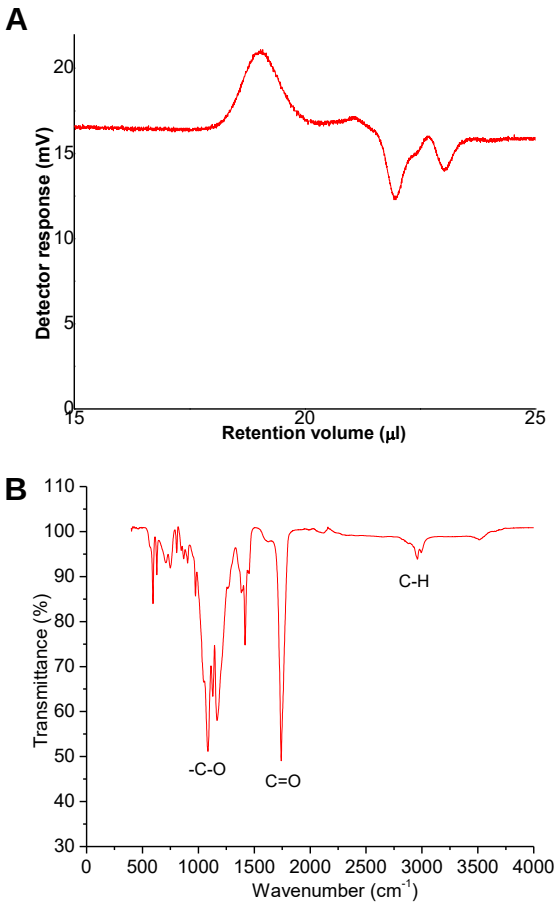

Figure S1. Characterization of mPEG-PLGA copolymer by gel permeation chromatography (GPC) (A) and ATR-FTIR spectrum (B). The molecular weight of mPEG-PLGA was determined by GPC in which mPEG-PLGA was dissolved in THF with respect to a polystyrene standard.

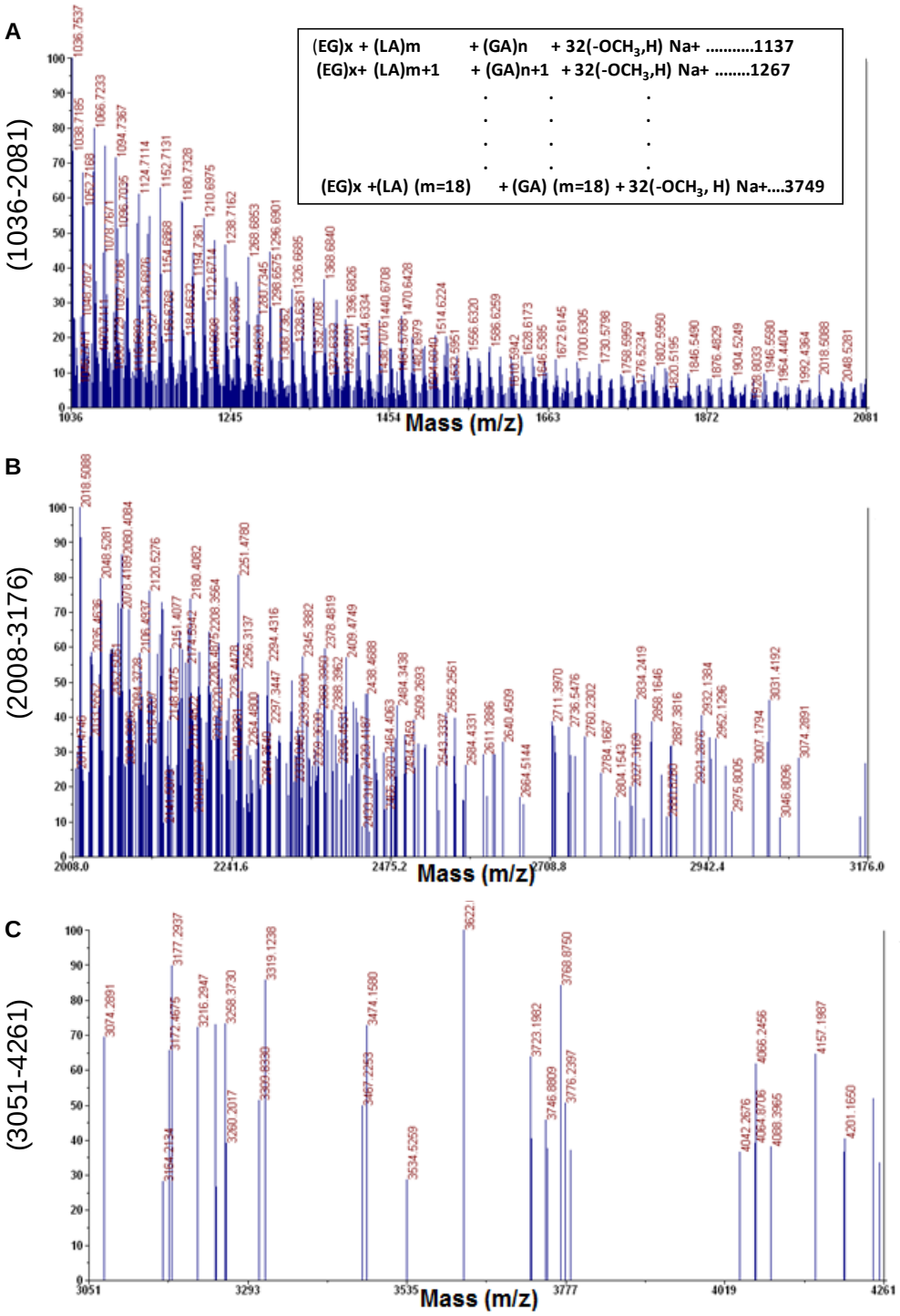

Figure S2. MALDI-TOF spectrum of mPEG-PLGA copolymers (A) Fragment of MALDI-TOF (1036-2081) **(B)** Fragment of MALDI-TOF **(2008-3176) (C)** Fragment of MALDI-TOF (3051-4261).

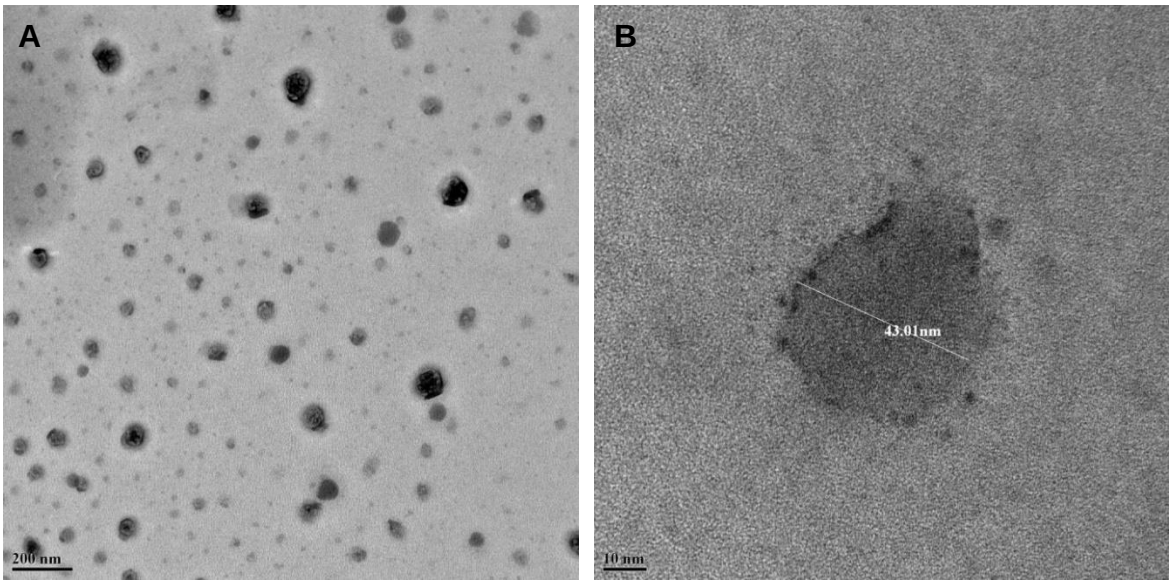

Figure S3. Transmission electron microscopy of P-NP-Ir. P-NP-Ir in aqueous suspension was dried on a carbon-coated copper TEM grid and stained with phosphotungstic acid. **(A)** Uniform nanoparticles (scale, 200nm) **(B)** Representative picture of single nanoparticle (scale 20 nm).

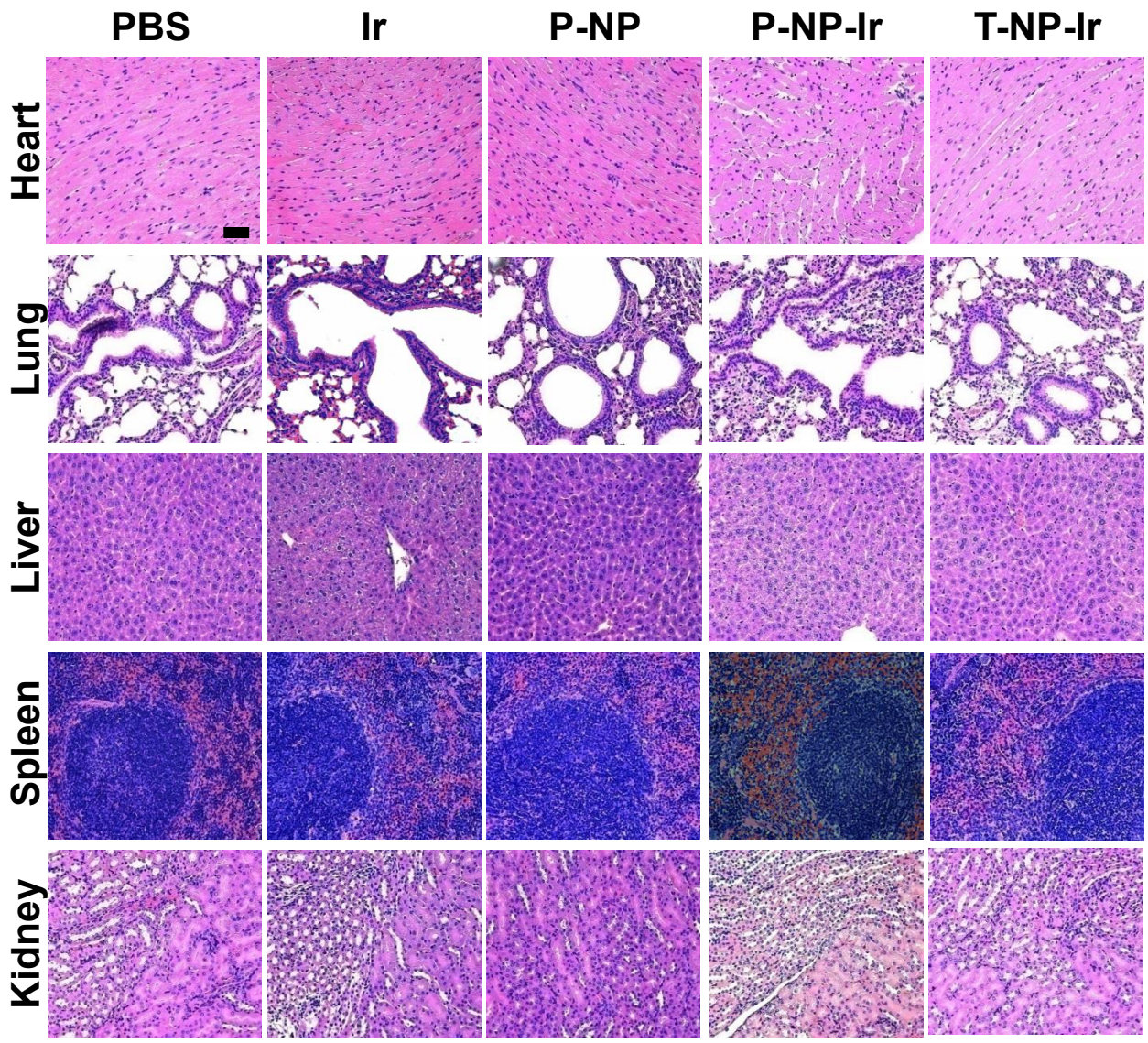

Figure S4. Histological examination of major organs after therapy studies. CT26 tumour-bearing Balb/c mice were intravenously administered of PBS (control), Ir (free drug), P-NP (empty NP), P-NP-Ir or T-NP-Ir. Each injection dose contained equivalent amount of Ir at 30 mg kg^{-1} and P-NP contained matching excipients weight in P-NP-Ir formulation. Mice received a total of 4 injections of the corresponding treatment on day 7, 11, 14 and 17 post-tumor inoculations and sacrificed on day 22. Major organs (heart, lung, liver, spleen and kidney) were excised, formalin-fixed, and stained with H&E. Scale bar, 50 μm.

Table S1. Optimization of drug loading in T-NPs

Formulation[a]	Initial drug (mg)	Hydrodynamic diameter (nm)[b,f]	PDI[b]	Zeta potential (mV) [c,f]	Encapsulation Efficiency (EE%)[d,f]	Loading Efficiency (LE%)[e,f]
T-NP	-	109 ± 8.2	0.317	-18.51 ± 1.6	N/A	N/A
T-NP-Ir	1	118.1 ± 2.7	0.369	-8.71±1.0	33.73 ±3.8	0.67 ±0.08
	3	125.9 ± 2.1	0.357	-9.1 ± 4.22	62.39 ± 3.4	3.7 ± 0.05
	5	163.8 ± 9.1	0.391	-10.3 ± 6.8	47.04 ± 5.6	4.74 ± 0.02

[a] PLGA 12.5 mg, soya lecithin 12.5 mg, ethanol 1 ml, acetone 1.5 ml, distilled water 5ml, Tween® 80 10mg.
[b] Measured by dynamic light scattering.
[c] Surface charge measured by electrophoresis.
[d] Calculated as percentage of initial drug added, determined by spectrophotometer.
[e] Calculated as mass of incorporated drug divided by the weight of polymer, determined by spectrophotometer.
[f] Expressed as mean ± SD (n=3).

Table S2. Optimization of drug loading in P-NPs

Formulation[a]	Initial drug (mg)	Hydrodynamic diameter (nm)[b,f]	PDI[b]	Zeta potential (mV) [c,f]	Encapsulation Efficiency (EE%)[d,f]	Loading Efficiency (LE%)[e,f]
P-NP	-	139.4 ± 2.7	0.321	-14.5 ± 5.2	N/A	N/A
P-NP-Ir	1	149.1 ± 2.9	0.234	-6.7 ± 1.0	38.30 ± 1.5	0.7 ± 0.05
	3	146.9 ± 8.8	0.256	-7.47 ± 6.8	65.75 ± 4.5	3.9 ± 0.08
	5	145.3 ± 7.3	0.261	-9.3 ± 5.1	57.73 ± 0.5	5.7 ± 0.02

[a] PLGA 12.5 mg, soya lecithin 12.5 mg, ethanol 1 ml, acetone 1.5 ml, distilled water 5ml, Pluronic®25mg.
[b] Measured by dynamic light scattering.
[c] Surface charge measured by electrophoresis.
[d] Calculated as percentage of initial drug added, determined by spectrophotometer.
[e] Calculated as mass of incorporated drug divided by the weight of polymer, determined by spectrophotometer.
[f] Expressed as mean ± SD (n=3).