Supporting information

Effect of crystallinity on the properties of polycaprolactone nanoparticles containing the dual FLAP/mPEGS-1 inhibitor BRP-187-187

Antje Vollrath [a, b], Christian Kretzer [c], Bärbel Beringer-Siemers [a], Blerina Shkodra [a, b], Justyna A. Czaplewska [a], Damiano Bandelli [a, b], Steffi Stumpf [a, b], Stephanie Hoeppener [a, b], Christine Weber [a, b], Oliver Werz [b, c], Ulrich S. Schubert [a, b] *

* Corresponding author

[a]Laboratory of Organic Chemistry and Macromolecular Chemistry (IOMC)
Friedrich Schiller University, Humboldtstraße 10, 07743 Jena, Germany

[b]Jena Center for Soft Matter (JCSM)
Friedrich Schiller University, Philosophenweg 7, 07743 Jena, Germany

[c]Department of Pharmaceutical/Medicinal Chemistry, Institute of Pharmacy
Friedrich Schiller University, Philosophenweg 14, 07743 Jena, Germany

Table S1. Molar mass and composition of the (co)polyesters. Details are described in a previous publication.[1]

εCL/ δCL[a] (mol %)	$M_{n,\,theo}$[b] (kg mol^{-1})	$M_{n,\,NMR}$[a] (kg mol^{-1})	$M_{n,\,SEC}$[c] (kg mol^{-1})	$Đ_{SEC}$ [c]
100/0	11	13	19	1.17
87/13	9	13	21	1.57
81/19	9	10	19	1.41
75/25	8	10	19	1.30
61/39	7	9	16	1.26
45/55	7	7	15	1.21
0/100	10	9	6	1.09

(a) Determined by ^{1}H-NMR spectroscopy of the purified polymers.
(b) Molar mass expected from monomer conversions and the feed ratio.
(c) Determined by size exclusion chromatography (eluent $CHCl_3$, refractive index detection, polystyrene calibration).

Supporting information

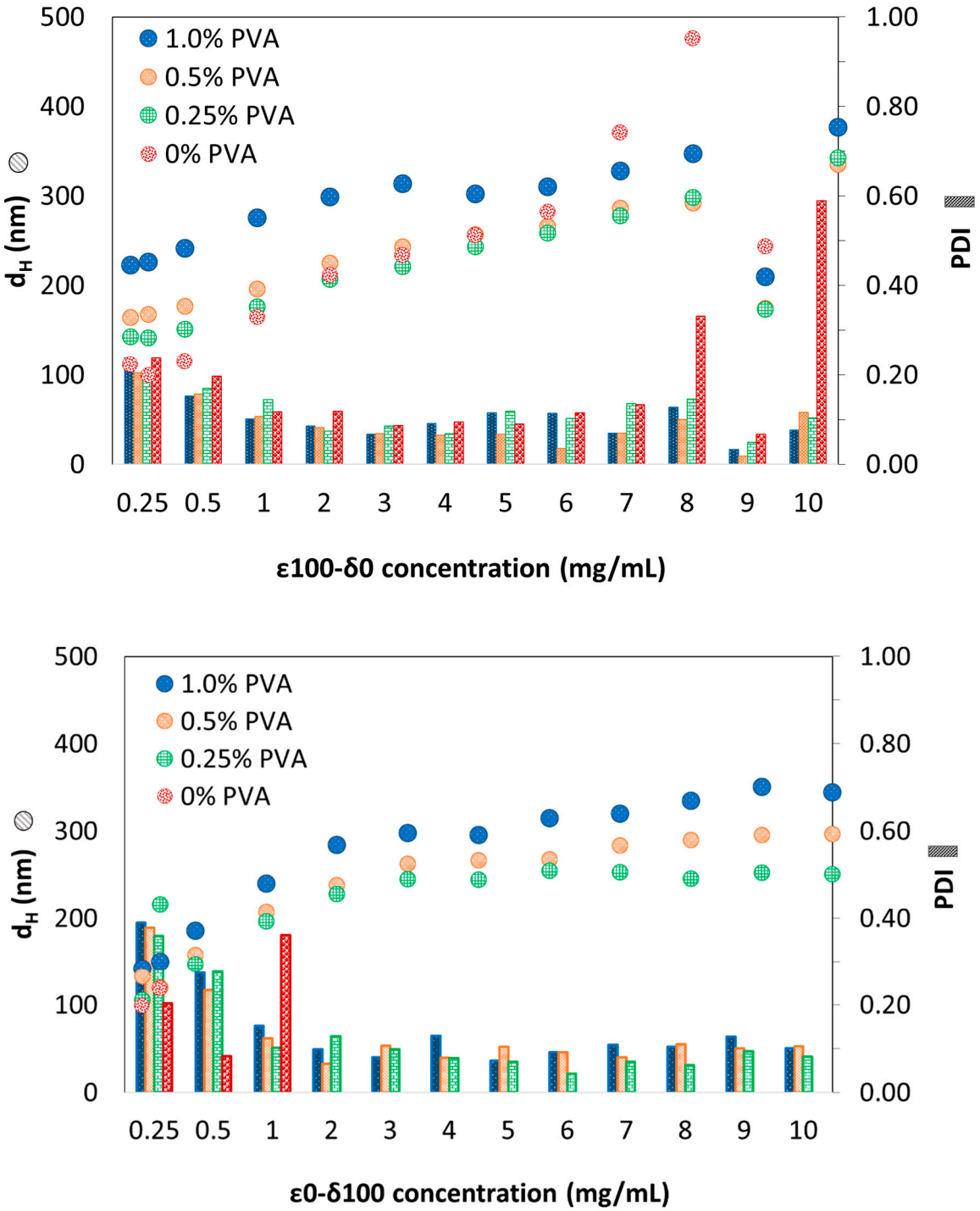


Figure S1. Hydrodynamic diameter (intensity-weighted distribution, circles) and PDI (bars) of the homopolymer ε100-δ0 and ε0-δ100 NPs over a range of PVA concentration used in the formulation.

Supporting information

Table S2. Properties of PCL[BRP-187] NPs formulated from THF utilizing polymer concentration of 5 mg mL^{-1} (n = 1 batch).

εCL/δCL (mol %)	Purified NPs[a]		Lyophilized NPs[b]		LC[c]	PVA[d]	Yield[e]
	d_H (nm)	PDI	d_H (nm)	PDI	(%, w/w)	(%)	
ε100-δ0	593	0.52	461	0.40	2.00	97	
ε87-δ13	427	0.23	349	0.30	2.01	86	
ε81-δ19	445	0.24	465	0.33	1.82	99	
ε75-δ25	421	0.28	651	0.62	1.88	95	
ε61-δ39	407	0.19	443	0.40	1.63	76	
ε45-δ55	397	0.26	414	0.36	2.26	60	
ε0- δ100	403	0.49	436	0.49	1.66	53	

d_H represents the intensity-weighted distribution.
(a) NPs measured after purification.
(b) NPs measured after lyophilization and subsequent resuspension in water.
(c) Determined by UV-VIS spectroscopy at λ = 316 nm (n = 4) and calculated using LC = (mass of drug recovered) / (mass of particle recovered) x 100.
(d) The determination of PVA in the NPs (%, w/w) was performed according to the published protocol.[15](e) Yield = (mass of NPs recovered including PVA residue) / (mass of polymer + mass of drug) in the formulation x 100.

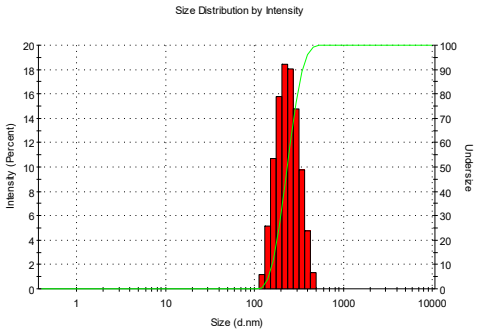
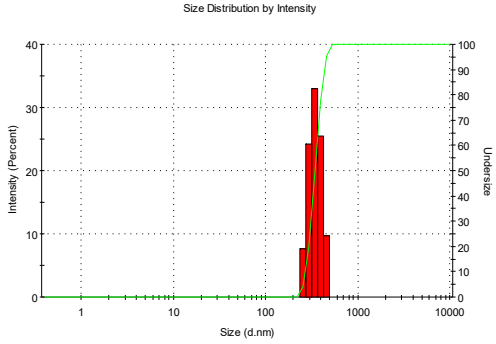
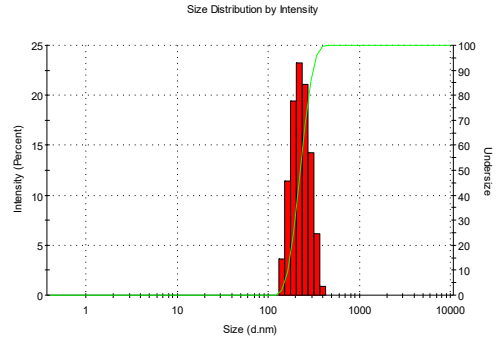
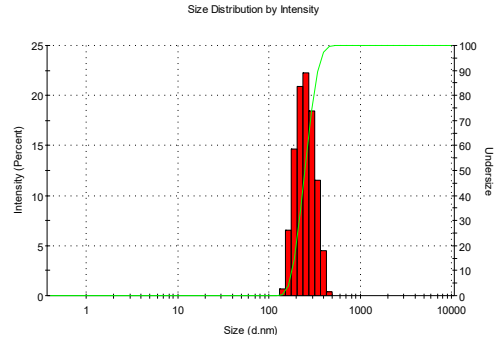
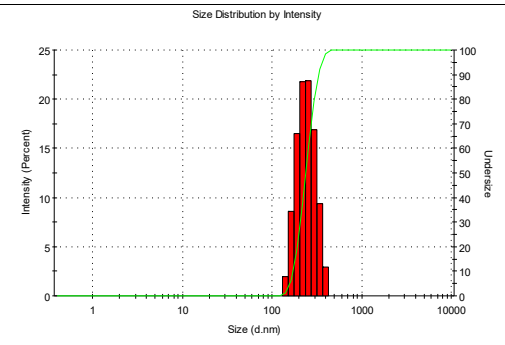
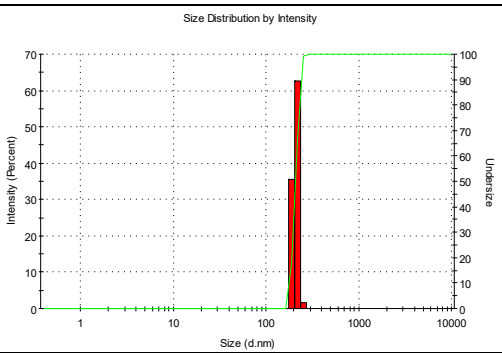
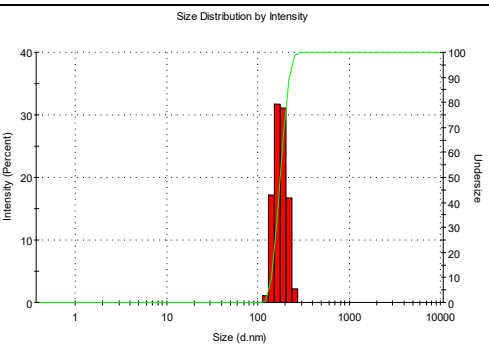
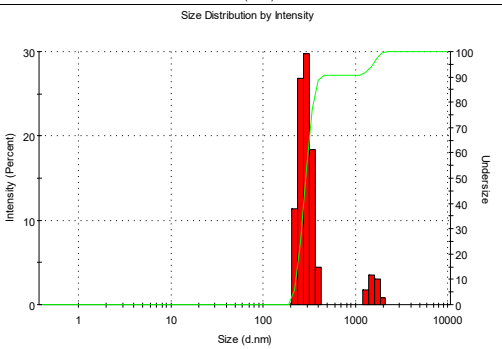
Table S3. Particle properties of empty PCL NPs prepared in THF with c = 2.5 mg mL^{-1} (n = 2 batches) obtained by DLS and ELS measurements after purification and after lyophilization and subsequent resuspension (n = 2 for purified NPs, n = 1 for lyophilized NPs).

εCL/δCL (mol %)	Purified NPs [a]		Lyophilized NPs [b]		ZP [b]	PVA [c]	Yield [d]
	d_H (nm)	PDI	d_H (nm)	PDI	(mV)	(%, w/w)	(%)
ε100-δ0	250	0.08	250	0.21	-33	2.0	93
ε87-δ13	177	0.05	253	0.27	-23	2.0	68
ε81-δ19	193	0.06	268	0.28	-38	1.8	70
ε75-δ25	196	0.05	279	0.33	-32	1.9	76
ε61-δ39	170	0.08	220	0.21	-13	1.6	54
ε45-δ55	163	0.06	205	0.23	-33	2.3	51
ε0- δ100	212	0.08	285	0.30	-40	1.7	67

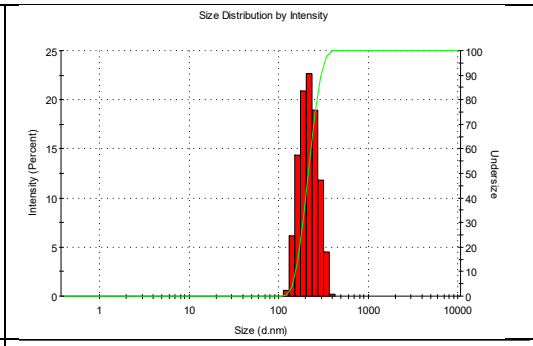
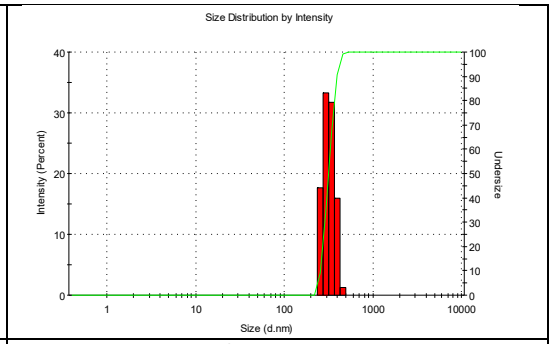
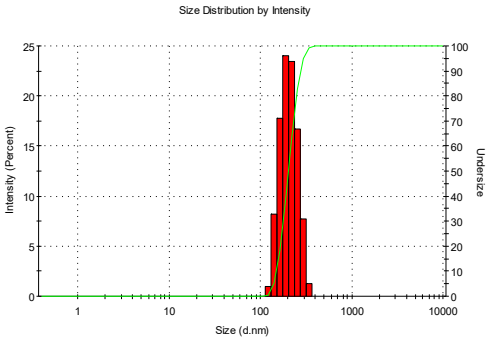
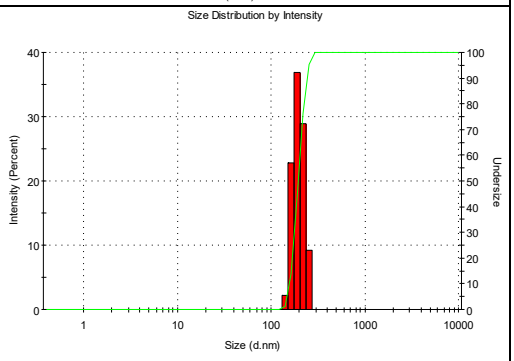
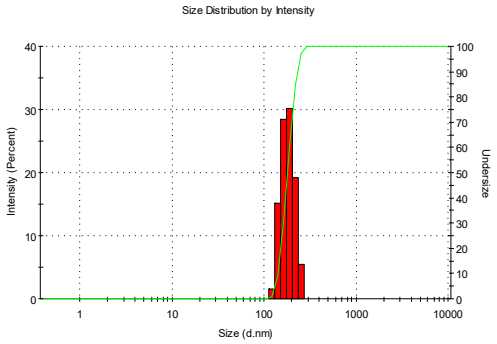
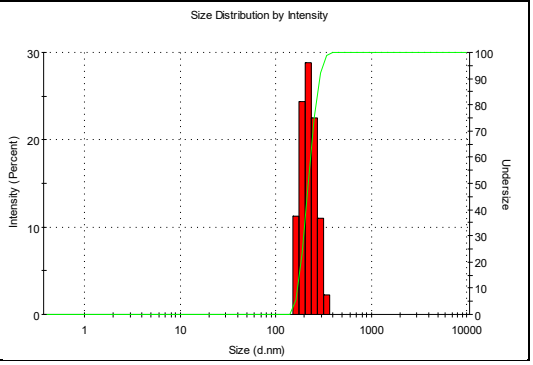
d_H represents the intensity-weighted distribution (five measurements) and zeta-potential (ZP) (three measurements).
(a) NPs measured after purification.
(b) NPs measured after lyophilization and subsequent resuspension in water.
(c) The determination of PVA in the NPs (%, w/w) was performed according to the published protocol.[15]
(d) Yield = (mass of NPs recovered – mass of found PVA) / (mass of polymer + mass of drug) in the formulation x 100.

Supporting information

Table S4. DLS intensity-weighted size distribution of PCL[BRP-187] NPs of one formulation round after purification, as well as after lyophilization and resuspension in water.

εCL/ δCL	After purification	After lyophilization & resuspension
ε100-δ0		
ε87-δ13		
ε81- δ19		
ε75- δ25		

Supporting information

ε61- δ39		
ε45- δ55		
ε0- δ100		

Supporting information

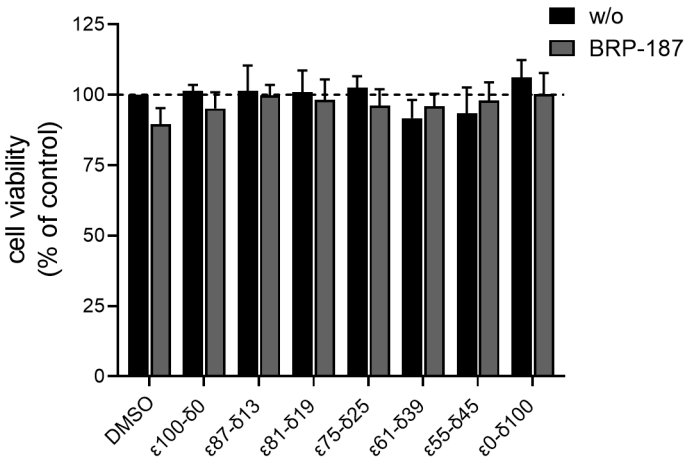


Figure S2. Cell viability measured with a Beckman ViCell XR cell counter by trypan blue staining. A total of 1 x 107 PMNL were diluted in PBS plus 0.1% of glucose and incubated with DMSO, BRP-187 (10 μM), empty PCL particles (labeled as w/o) or PCL particles with BRP-187 (labeled with BRP-187; respective amount to 10 μM BRP-187) for 5 h at 37 °C. Values are given as 5-LO products as a percentage of control (DMSO) (n = 3).

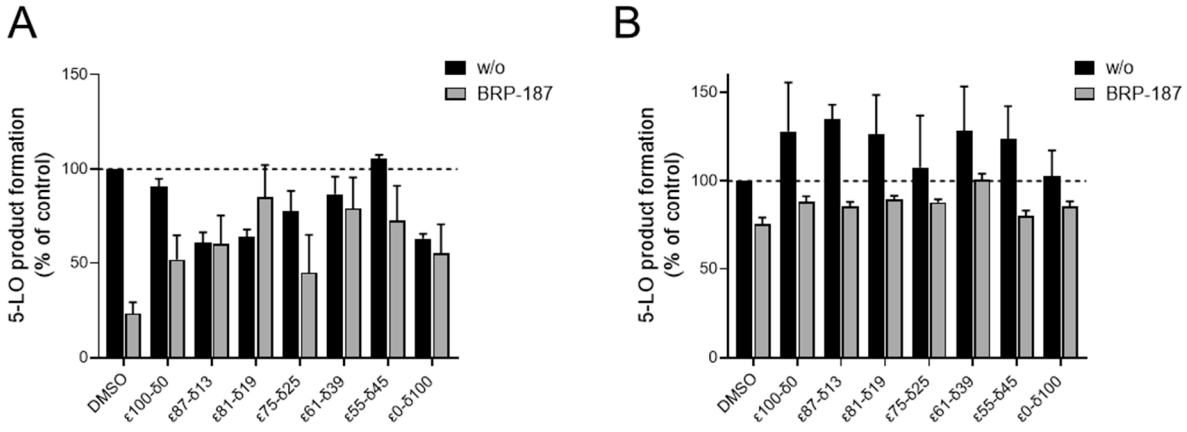


Figure S3. Measurement of 5-LO product formation as indicator for the inhibition of the drug target 5-lipoxygenase-activating protein (FLAP) by BRP-187.[37] A total of 5 x 106 polymorphonuclear leukocytes (PMNL) diluted in PBS containing 0.1% glucose and 1mM $CaCl_2$ were preincubated with DMSO, BRP-187 (0.3 μM), empty PCL particles (labeled as w/o) or PCL particles with BRP-187 (labeled as BRP-187; 0.3 μM respective BRP-187) for 1 h (A) and 2 h (B) at 37 °C and further stimulated with 2.5 μM A23187 for 10 min. The reaction was stopped with 1 mL ice-cold methanol containing 200 ng mL^{-1} PGB1 as internal standard. Lipid mediators were extracted via solid-phase extraction (SPE) and analyzed with HPLC. Values are given as 5-LO products (LTB_4, its trans-isomers [4] and 5-HETE) as a percentage of control (DMSO) (n = 3).

Supporting information

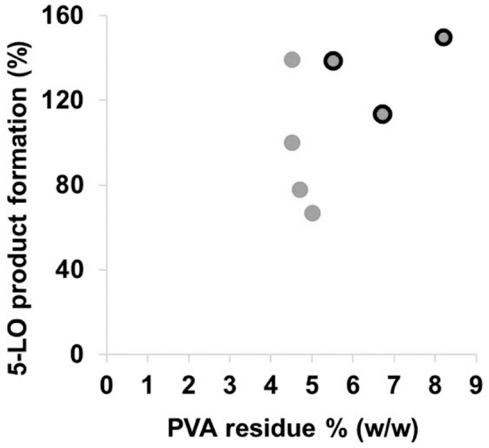


Figure S4. Influence of the residual PVA on the efficiency of drug-loaded PCL NPs on 5-LO inhibition. Black-circled data points represent PCL polymers with bulk degree of crystallinity below 10% and glass transition temperature $T_g < 37$ °C.