

Electronic Supplementary Information (ESI)

**Zapped Assembly of Polymeric (ZAP) Nanoparticles for Anti-Cancer Drug  
Delivery**

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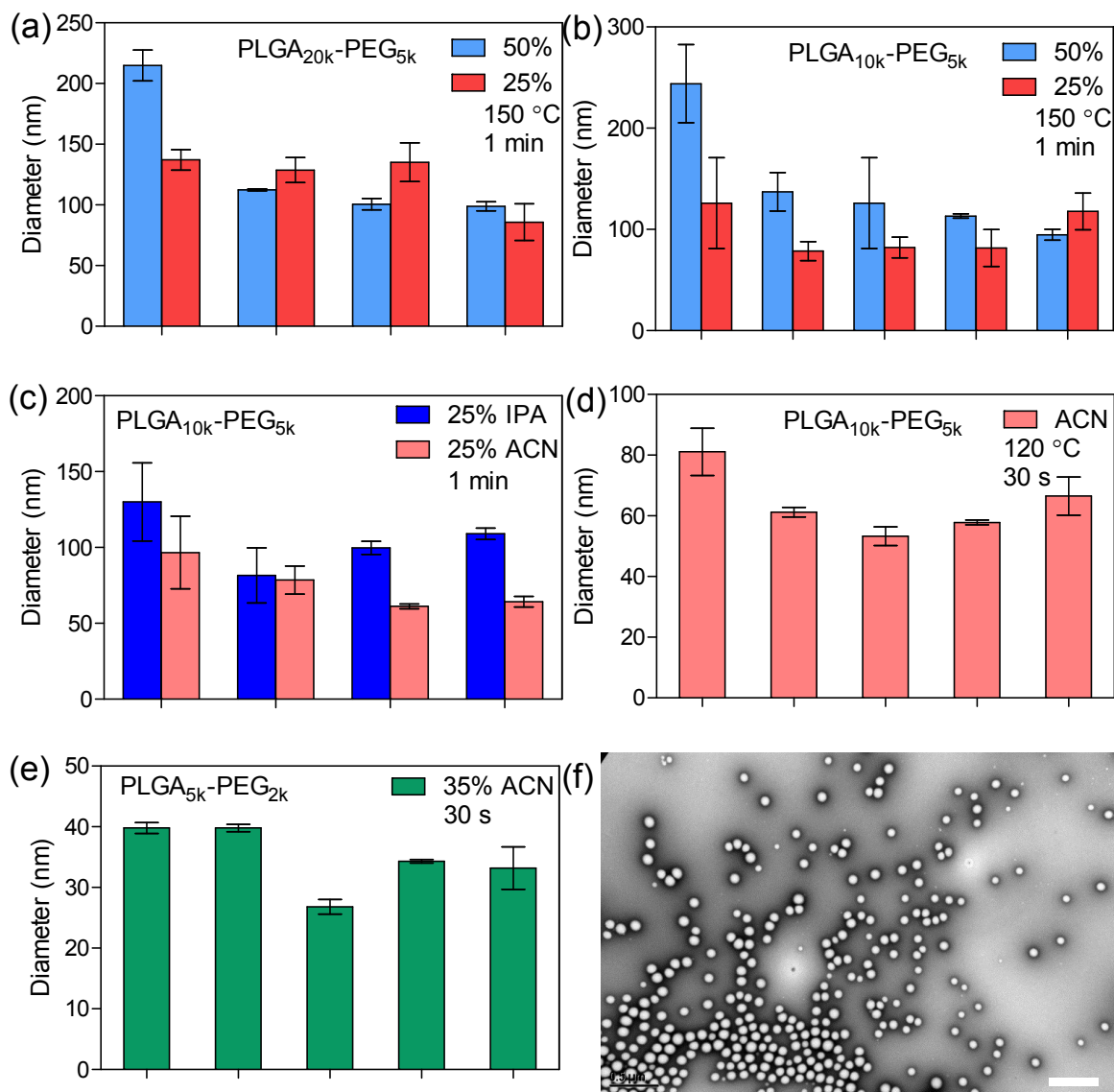
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**Figure S1.** (a)-(e) Nanoparticle diameters of systematically screened ZAP NPs prepared from different PLGA-PEG copolymers, solvents (weight percentages in water), and reaction parameters. (f) TEM image of ZAP PLGA<sub>20k</sub>-PEG<sub>5k</sub> NPs (scale bar = 0.5 μm). DMF = N,N-dimethylformamide; Ace = Acetone; ACN = Acetonitrile; IPA = Isopropanol.

**Table S1.** Nanoparticle diameters of ZAP PLGA<sub>20k</sub>-PEG<sub>5k</sub> NPs prepared at 150 °C in 1 min using different solvents.

Organic Solvent	Solvent weight percent in water	Diameter (nm)
N,N-dimethylformamide	50	214.8 ± 12.7
N,N-dimethylformamide	25	137.1 ± 8.5
Acetone	50	112.4 ± 0.9
Acetone	25	128.7 ± 10.3
Acetonitrile	50	100.6 ± 4.7

Acetonitrile	25	135.2 ± 16.0
Isopropanol	50	98.8 ± 3.7
Isopropanol	25	85.8 ± 15.1

**Table S2.** Nanoparticle diameters of ZAP PLGA<sub>10k</sub>-PEG<sub>5k</sub> NPs prepared at 150 °C in 1 min using different solvents.

Organic Solvent	Solvent weight percent in water	Diameter (nm)
N,N-dimethylformamide	50	243.9 ± 38.6
N,N-dimethylformamide	25	125.9 ± 44.9
Acetonitrile	50	137.1 ± 19.0
Acetonitrile	25	78.5 ± 9.3
Ethanol	50	125.9 ± 44.9
Ethanol	25	82.1 ± 10.2
Isopropanol	50	113.2 ± 2.2
Isopropanol	25	81.6 ± 18.2
Acetone	50	94.7 ± 5.3
Acetone	25	117.8 ± 18.1

**Table S3.** Nanoparticle diameters of ZAP PLGA<sub>10k</sub>-PEG<sub>5k</sub> NPs prepared at different temperatures in 1 min using different solvents (25 wt% organic in water).

Temperature (°C)	Organic Solvent	Diameter (nm)
180	Isopropanol	130.0 ± 25.8
180	Acetonitrile	96.6 ± 23.9
150	Isopropanol	81.6 ± 18.2
150	Acetonitrile	78.5 ± 9.3
120	Isopropanol	99.7 ± 4.3
120	Acetonitrile	61.2 ± 1.6
90	Isopropanol	109.0 ± 3.8
90	Acetonitrile	64.3 ± 3.5

**Table S4.** Nanoparticle diameters of ZAP PLGA<sub>10k</sub>-PEG<sub>5k</sub> NPs prepared at 120 °C in 30 sec using different weight percentages of acetonitrile in water.

Acetonitrile weight percent in water	Diameter (nm)
15	81.1 ± 7.8
25	61.2 ± 1.6
35	53.3 ± 3.1
43	57.8 ± 0.8
50	66.5 ± 6.3

**Table S5.** Nanoparticle diameters of ZAP PLGA<sub>5k</sub>-PEG<sub>2k</sub> NPs prepared at different temperatures in 30 sec using 35 wt% acetonitrile in water.

Temperature (°C)	Diameter (nm)
60	39.8 ± 0.9
75	39.8 ± 0.6
90	26.8 ± 1.2
105	34.3 ± 0.3
120	33.2 ± 3.5

**ZAP NP Reaction Parameter Code:**

*PLGA-PEG:* 10k-5k (A), 20k-5k (B), 55k-5k (C), 5k-2k (D)

*Time:* 30 s (1), 1 min (2), 5 min (3), 30 min (4), 1 h (5), 2 h (6), 1 s (7)

*Temp (°C):* 90 (D), 120 (E), 150 (F), 180 (G), 105 (H), 75 (I), 60 (J)

*Solvent:* water (H), acetonitrile (I), acetone (J), 2-propanol (K), ethanol (L), N,N-dimethylformamide (M), dimethylsulfoxide (N), tetrahydrofuran (O)

*Wi% organic solvent:* 0, 15, 25, 35, 43, 50

**Table S6.** DLS analysis of ZAP PLGA-PEG NP library with corresponding reaction code including Z-average diameter ( $D_z$ ), polydispersity index (PDI), and PDI width.

Number	$D_z$ (nm)		PDI		PDI width (nm)		Reaction code
1	348.1	± 10.6	0.13	± 0.02	124.9	± 11.6	C6FK25
2	342.1	± 38.9	0.30	± 0.10	183.4	± 14.3	C6FO25
3	302.3	± 58.2	0.24	± 0.16	135.8	± 18.8	C6FK35
4	266.4	± 23.1	0.31	± 0.04	147.3	± 17.9	C6DJ25
5	255.8	± 43.2	0.29	± 0.13	132.7	± 8.7	C6GJ15
6	243.9	± 38.6	0.14	± 0.01	90.0	± 11.9	A2FM50
7	243.8	± 37.0	0.19	± 0.10	102.2	± 45.8	C6FI35
8	214.8	± 12.7	0.14	± 0.04	80.2	± 14.0	B2FM50
9	212.7	± 39.5	0.41	± 0.14	136.4	± 42	B3FH0
10	209.0	± 18.1	0.16	± 0.03	84.1	± 14.8	C6FI25
11	208.3	± 10.0	0.25	± 0.07	102.3	± 10.3	C6GJ25
12	204.3	± 0.9	0.12	± 0.02	69.8	± 7.1	C6FJ35
13	203.3	± 26.5	0.07	± 0.01	51.7	± 10.4	B2FL50
14	199.6	± 14.7	0.32	± 0.09	112.0	± 23.6	B2FH0
15	197.8	± 24.5	0.12	± 0.04	69.3	± 16.5	A6FJ25
16	197.2	± 24.9	0.19	± 0.07	86.8	± 26.6	C6FK15
17	193.9	± 38.9	0.30	± 0.18	107.1	± 54.8	C6FJ15
18	191.4	± 25.2	0.17	± 0.03	78.2	± 7.0	C6GJ35
19	179.0	± 43.3	0.18	± 0.05	74.8	± 16.8	C6EJ25
20	178.8	± 7.8	0.15	± 0.04	68.8	± 9.2	B6FJ25
21	178.5	± 8.7	0.21	± 0.02	82.2	± 2.2	B3FO25
22	171.9	± 7.3	0.17	± 0.04	70.7	± 6.7	B4FJ25

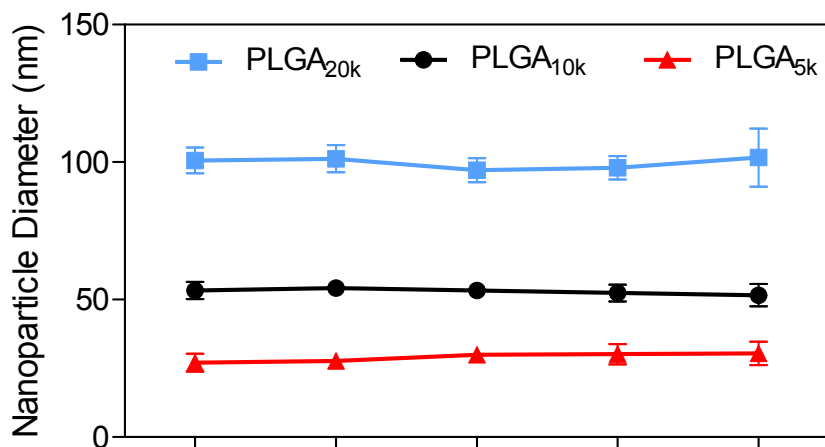
23	166.7	±	18.5	0.11	±	0.01	54.3	±	9.1	A3FJ25
24	164.8	±	14.8	0.09	±	0.02	50.6	±	10.0	B5FJ25
25	155.7	±	8.4	0.19	±	0.08	65.3	±	12.3	A5FJ25
26	142.1	±	10.8	0.15	±	0.05	54.1	±	9.0	B3GJ25
27	139.6	±	32.8	0.22	±	0.05	66.2	±	23.4	B3FJ15
28	138.2	±	9.2	0.14	±	0.07	51.2	±	14.6	C6FJ25
29	137.9	±	5.9	0.27	±	0.09	71.8	±	15.5	B3EJ25
30	137.1	±	8.5	0.17	±	0.01	55.7	±	4.1	B2FM25
31	137.1	±	19.0	0.13	±	0.02	49.4	±	8.7	A2FI50
32	135.2	±	16.0	0.24	±	0.07	67.0	±	18.2	B2FI25
33	133.4	±	12.3	0.13	±	0.01	48.3	±	6.5	B3FM25
34	133.1	±	3.4	0.22	±	0.04	62.4	±	7.2	B2DJ25
35	132.5	±	8.3	0.19	±	0.04	57.2	±	9.4	B3FJ25
36	130.0	±	25.8	0.14	±	0.07	46.5	±	2.1	A2GJ25
37	128.7	±	13.2	0.15	±	0.04	50.0	±	10.7	B3FJ35
38	128.7	±	10.3	0.19	±	0.04	55.9	±	7.0	B2FJ25
39	128.7	±	9.0	0.25	±	0.04	63.8	±	8.6	B2EJ25
40	126.0	±	17.4	0.17	±	0.02	51.3	±	4.6	B3FJ50
41	124.4	±	6.2	0.19	±	0.01	53.8	±	2.7	B3FI25
42	123.5	±	6.0	0.20	±	0.04	54.6	±	7.4	B2GJ25
43	117.8	±	18.1	0.16	±	0.06	44.9	±	2.5	A2FJ25
44	113.2	±	2.2	0.21	±	0.05	51.5	±	7.3	A2FK50
45	112.4	±	0.9	0.12	±	0.03	38.1	±	5.4	B2FJ50
46	109.0	±	3.8	0.23	±	0.01	52.4	±	0.7	A2DJ25
47	108.0	±	10.3	0.13	±	0.02	39.3	±	7.1	B2GK25
48	100.6	±	4.7	0.15	±	0.03	38.8	±	5	B2FI50
49	99.7	±	4.3	0.27	±	0.04	51.6	±	4.5	A2EJ25
50	98.8	±	3.7	0.09	±	0.01	28.7	±	1.2	B2FK50
51	94.8	±	1.5	0.15	±	0.03	37.0	±	4.6	B2DK25
52	94.8	±	6.4	0.11	±	0.05	31.0	±	5.2	A2FK15
53	94.7	±	5.3	0.19	±	0.03	41.4	±	2.6	A2FJ50
54	85.8	±	15.1	0.06	±	0.01	20.3	±	2.6	B2FK25
55	82.1	±	10.2	0.26	±	0.03	41.8	±	2.7	A2FL25
56	81.6	±	18.2	0.14	±	0.01	30.7	±	7.9	A2FK25
57	81.1	±	7.8	0.23	±	0.03	39.2	±	5.6	A1EI15
58	80.9	±	1.9	0.08	±	0.02	22.7	±	2.7	B2EK25
59	78.5	±	9.3	0.22	±	0.11	35.5	±	9.4	A2FI25
60	68.7	±	9.3	0.16	±	0.07	27.3	±	10.2	A1FI25
61	66.5	±	6.3	0.17	±	0.02	27.7	±	3.4	A1EI50
62	64.3	±	3.5	0.16	±	0.05	25.2	±	5.5	A1DI25
63	63.2	±	1.6	0.13	±	0.06	22.1	±	5.7	A1EI25
64	62.4	±	0.8	0.10	±	0.03	19.3	±	2.8	A2EI25

65	53.3	±	3.1	0.13	±	0.06	18.9	±	5.4	A1EI35
66	50.0	±	3.3	0.26	±	0.02	25.6	±	2.8	D1EL25
67	44.6	±	1.2	0.22	±	0.02	20.8	±	1.3	D1EK25
68	43.1	±	9.9	0.22	±	0.05	20.2	±	7.0	D1EK15
69	43.0	±	1.0	0.29	±	0.05	23.1	±	2.6	D1EI15
70	41.9	±	1.9	0.20	±	0.03	18.8	±	2.3	D1DI50
71	40.7	±	3.0	0.07	±	0.01	10.6	±	0.8	D2FI50
72	39.8	±	0.9	0.33	±	0.04	23.0	±	1.7	D1JI35
73	39.8	±	0.6	0.33	±	0.01	23.0	±	0.2	D1II35
74	39.4	±	1.1	0.34	±	0.01	22.8	±	0.9	D7DI35
75	38.2	±	1.9	0.26	±	0.04	19.5	±	2.2	D1EI25
76	36.3	±	1.8	0.29	±	0.02	19.4	±	1.6	D2DI35
77	36.1	±	4.8	0.20	±	0.06	16.0	±	4.5	D1DI43
78	34.3	±	0.3	0.25	±	0.02	17.0	±	0.6	D1HI35
79	33.2	±	3.5	0.19	±	0.02	14.4	±	1.9	D1EI35
80	29.7	±	1.4	0.20	±	0.03	13.2	±	1.2	D1DK25
81	28.7	±	0.7	0.21	±	0.02	13.2	±	0.8	D1EK25
82	28.2	±	1.4	0.18	±	0.08	11.7	±	3.3	D1EK50
83	27.6	±	1.1	0.12	±	0.04	9.5	±	1.1	D1EK35
84	26.8	±	1.2	0.26	±	0.05	13.7	±	1.7	D1DI35

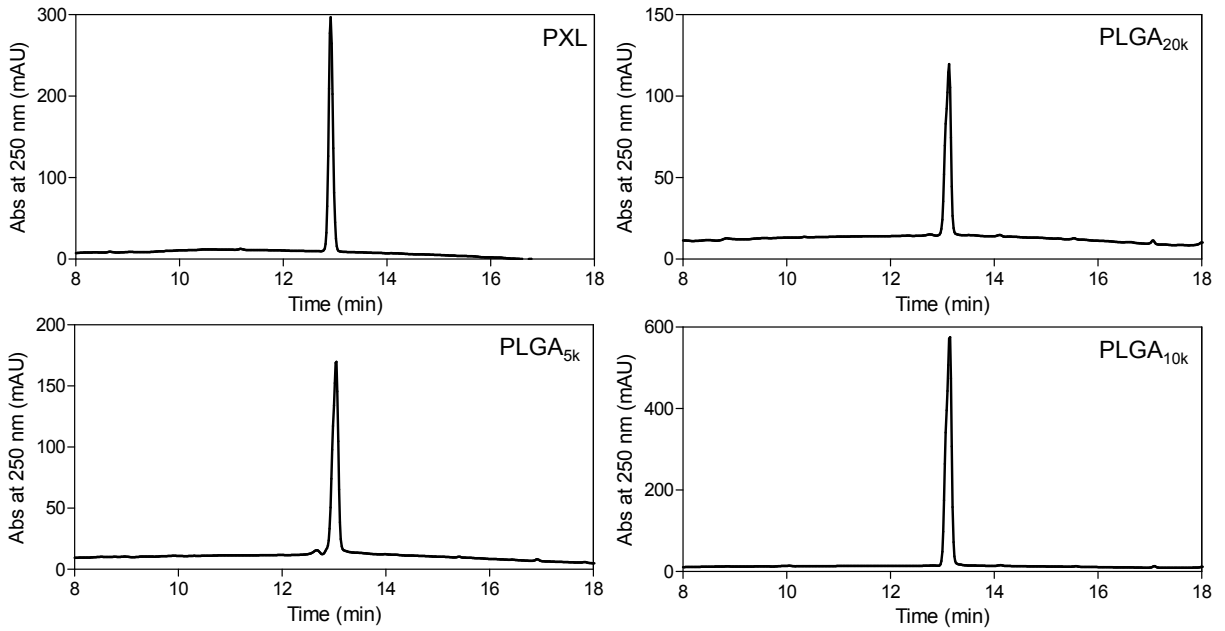
**Table S7.** Reaction code for ZAP PLGA-PEG NPs in Table 1 and Figure 1 inset.

Polymer	Reaction code	Nanoparticle diameter <sup>a</sup> (nm)
PLGA <sub>5k</sub> -PEG <sub>2k</sub>	D1DI35	18.2 ± 1.8
PLGA <sub>10k</sub> -PEG <sub>5k</sub>	A1EI35	32.3 ± 2.3
PLGA <sub>20k</sub> -PEG <sub>5k</sub>	B2FI50	72.4 ± 7.4

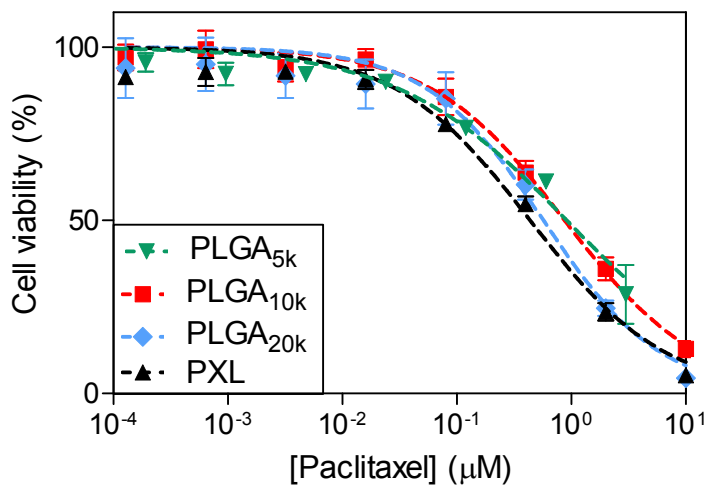
<sup>a</sup>Number-average nanoparticle diameter



**Figure S2.** Diameters of ZAP NPs in one week intervals over time in water at 23 °C.



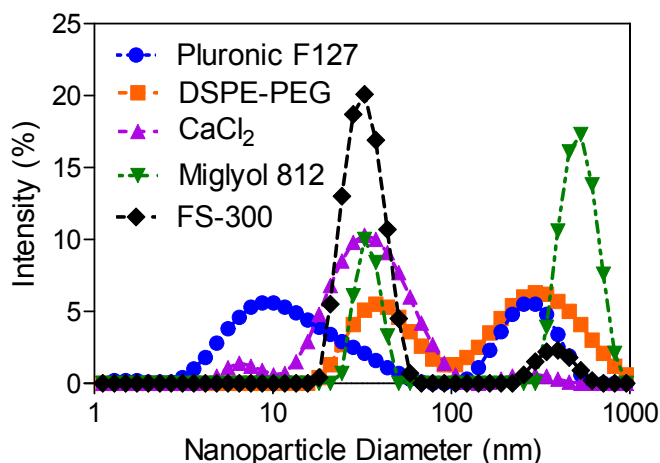
**Figure S3.** HPLC traces of native paclitaxel (PXL) and nanoparticle encapsulated PXL using PLGA<sub>20k</sub>, PLGA<sub>10k</sub>, and PLGA<sub>5k</sub>, which shows that the integrity of PXL was maintained using the ZAP NP fabrication process.



**Figure S4.** Percent viability of HeLa cells dosed with paclitaxel and paclitaxel-loaded nanoparticles at different doses for 4 h followed by 68 h incubation.







**Figure S7.** Size distribution for aggregated and polydisperse samples of ZAP NPs with different additives.

**Table S8.** Compositions of ZAP PLGA<sub>5k</sub>-PEG<sub>2k</sub>, PLGA<sub>33k</sub>, and surfactant additive NPs in weight percent.

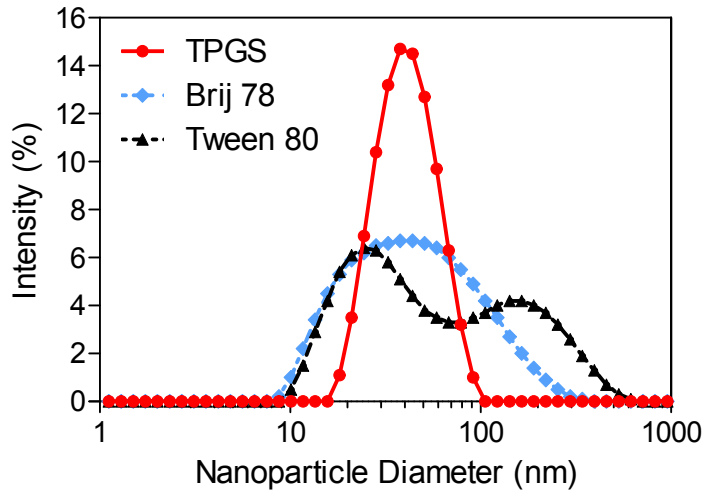
Composition	PLGA <sub>5k</sub> -PEG <sub>2k</sub>	PLGA <sub>33k</sub>	Surfactant
A	55.6 %	11.1 %	33.3 %
B	66.7 %	8.3 %	25.0 %
C	77.8 %	5.5 %	16.7 %

**Table S9.** DLS characterization of ZAP PLGA<sub>5k</sub>-PEG<sub>2k</sub>, PLGA<sub>33k</sub>, and surfactant additive NPs with composition A, B, or C listed in Table S8.

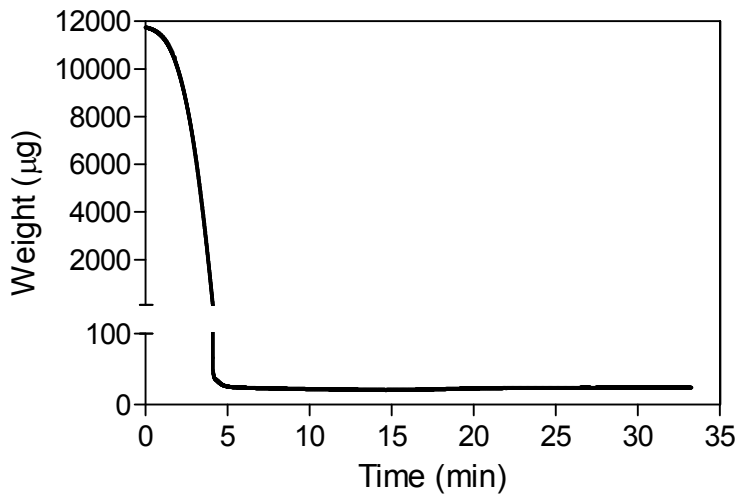
Sample	D <sub>z</sub> (nm)	PDI	Width (nm)
Brij78, A	51.5 ± 1.4	0.18 ± 0.03	22.0 ± 2.3
Brij78, B	45.2 ± 4.0	0.20 ± 0.04	20.2 ± 3.7
Brij78, C	40.7 ± 3.3	0.22 ± 0.02	19.0 ± 2.1
TPGS, A	60.7 ± 12.1	0.29 ± 0.05	32.8 ± 8.8
TPGS, B	44.7 ± 5.2	0.20 ± 0.04	19.8 ± 2.2
TPGS, C	41.4 ± 2.4	0.17 ± 0.04	17.0 ± 3.1
Tween 80, A	75.6 ± 22.3	0.35 ± 0.16	46.3 ± 22.8
Tween 80, B	43.8 ± 2.3	0.20 ± 0.03	19.4 ± 2.5
Tween 80, C	40.8 ± 2.2	0.23 ± 0.03	19.3 ± 1.8

**Table S10.** DLS characterization of 15 wt% charged PXL in ZAP PLGA-based NPs with additives.

Additive	D <sub>z</sub> (nm)	PDI	PDI Width (nm)
TPGS	38.8 ± 2.0	0.24 ± 0.03	19.0 ± 2.0
Tween 80	35.2 ± 3.9	0.37 ± 0.05	21.2 ± 1.4
Brij 78	49.3 ± 18.2	0.51 ± 0.06	35.4 ± 14.5



**Figure S8.** Size distribution for ZAP PLGA-based NPs with Brij 78, Tween 80, and TPGS additives charged with 15 wt% PXL.



**Figure S9.** Representative TGA thermogram for paclitaxel-loaded ZAP PLGA<sub>5k</sub>-PEG<sub>2k</sub> NPs. The TGA method was: ramp at 30 °C/min to 150 °C; hold isothermal for 10 minutes; ramp at 20 °C/min to 25 °C; hold isothermal for 2 minutes. Nanoparticle concentration was determined by dividing the weight at the end of the run by the volume used to aliquot the NP dispersion into the pan.