

*Research article***Chemotherapeutic loading via tailoring of drug-carrier interactions in poly (sialic acid) micelles**Gerald Pawlish<sup>1</sup>, Kyle Spivack<sup>2</sup>, Adam Gabriel<sup>2</sup>, Zuyi Huang<sup>2</sup> and Noelle Comolli<sup>2,\*</sup><sup>1</sup> Department of Bioengineering, College of Engineering, Temple University, 1947 N 12<sup>th</sup> St, Philadelphia, PA 19122, USA<sup>2</sup> Department of Chemical Engineering, College of Engineering, Villanova University, 800 E. Lancaster Ave., Villanova, PA 19085, USA\* **Correspondence:** Email: Noelle.Comolli@villanova.edu; Tel: +6105197134.**Supplementary****Table S1.** <sup>1</sup>H-NMR peak shifts of PSA in D<sub>2</sub>O.

Group	δ ppm	Letter	Group	δ ppm	Letter
HO-CH <sub>2</sub> -CHOH-CHOH-C			HO-CH <sub>2</sub> -CHOH- <u>CHOH-C</u>		
HO-C <sub>2</sub> O <sub>2</sub> Na-CH <sub>2</sub> - <u>CHOH-</u> CH-CONH-CH <sub>3</sub>	4.18	c	<u>HO</u> -C <sub>2</sub> O <sub>2</sub> Na-CH <sub>2</sub> -CHOH-C	3.65	d f h'
HO-CH <sub>2</sub> -CHOH-CHOH-C			H-CONH-CH <sub>3</sub>		
HO-C <sub>2</sub> O <sub>2</sub> Na-CH <sub>2</sub> -CHOH- <u>CH</u> -CONH-CH <sub>3</sub>	4.11	e	HO-CH <sub>2</sub> -CHOH-CHOH-C		
HO-CH <sub>2</sub> - <u>CHOH</u> -CHOH-C			HO-C <sub>2</sub> O <sub>2</sub> Na- <u>CH<sub>2</sub></u> -CHOH-C	2.68	a
HO-C <sub>2</sub> O <sub>2</sub> Na-CH <sub>2</sub> -CHOH- CH-CONH-CH <sub>3</sub>	3.90	g	H-CONH-CH <sub>3</sub>		
HO- <u>CH<sub>2</sub></u> -CHOH-CHOH-C			HO-CH <sub>2</sub> -CHOH-CHOH-C		
HO-C <sub>2</sub> O <sub>2</sub> Na-CH <sub>2</sub> -CHOH- CH-CONH-CH <sub>3</sub>	3.83	h	HO-C <sub>2</sub> O <sub>2</sub> Na-CH <sub>2</sub> -CHOH-C	2.09	i
HO- <u>CH<sub>2</sub></u> -CHOH-CHOH-C			H-CONH- <u>CH<sub>3</sub></u>		
HO-C <sub>2</sub> O <sub>2</sub> Na-CH <sub>2</sub> -CHOH- CH-CONH-CH <sub>3</sub>			HO-CH <sub>2</sub> -CHOH-CHOH-C		
			HO-C <sub>2</sub> O <sub>2</sub> Na- <u>CH<sub>2</sub></u> -CHOH-C	1.75	b
			H-CONH-CH <sub>3</sub>		

**Table S2.** <sup>1</sup>H-NMR peak shifts of PSA + EDC/NHS in D<sub>2</sub>O.

Group	δ ppm	Letter	Group	δ ppm	Letter
CH <sub>3</sub> CH <sub>3</sub> N-CH <sub>2</sub> -CH <sub>2</sub> -C			<u>CH<sub>3</sub>CH<sub>3</sub></u> N-CH <sub>2</sub> -CH <sub>2</sub> -C		
H <sub>2</sub> -NHCO-CH <sub>3</sub> <u>CH<sub>2</sub></u> N-C	3.20	g	H <sub>2</sub> -NHCO-CH <sub>3</sub> CH <sub>2</sub> N-C	2.90	e
O-PSA			O-PSA		
CH <sub>3</sub> CH <sub>3</sub> N-CH <sub>2</sub> -CH <sub>2</sub> - <u>C</u>			CH <sub>3</sub> CH <sub>3</sub> N-CH <sub>2</sub> - <u>CH<sub>2</sub></u> -C		
<u>H<sub>2</sub></u> -NHCO-CH <sub>3</sub> CH <sub>2</sub> N-C	3.15	h	H <sub>2</sub> -NHCO-CH <sub>3</sub> CH <sub>2</sub> N-C	1.90	i
O-PSA			O-PSA		
CH <sub>3</sub> CH <sub>3</sub> N- <u>CH<sub>2</sub></u> -CH <sub>2</sub> -C			CH <sub>3</sub> CH <sub>3</sub> N-CH <sub>2</sub> -CH <sub>2</sub> -C		
H <sub>2</sub> -NHCO-CH <sub>3</sub> CH <sub>2</sub> N-C	3.10	k	H <sub>2</sub> -NHCO- <u>CH<sub>3</sub></u> CH <sub>2</sub> N-C	1.09	f
O-PSA			O-PSA		

**Table S3.** <sup>1</sup>H-NMR peak shifts of PEA-g-PSA in D<sub>2</sub>O.

Group	δ ppm	Letter	Group	δ ppm	Letter
PSA-NH-CH <sub>2</sub> -CH <sub>2</sub> - <u>C<sub>6</sub></u>	7.44	d <sub>1</sub>	PSA-NH- <u>CH<sub>2</sub></u> -CH <sub>2</sub> -C <sub>6</sub>	3.02	a <sub>1</sub>
<u>H<sub>5</sub>-META</u>			H <sub>5</sub>		
PSA-NH-CH <sub>2</sub> -CH <sub>2</sub> - <u>C<sub>6</sub></u>	7.36	c <sub>1</sub> /e <sub>1</sub>	PSA-NH-CH <sub>2</sub> - <u>CH<sub>2</sub></u> -C <sub>6</sub>	2.78	b <sub>1</sub>
<u>H<sub>5</sub>-ORTHO/PARA</u>			H <sub>5</sub>		

**Table S4.** <sup>1</sup>H-NMR peak shifts of PPA-g-PSA in D<sub>2</sub>O.

Group	δ ppm	Letter	Group	δ ppm	Letter
PSA-NH-CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> - <u>C<sub>6</sub>H<sub>5</sub>-META</u>	7.40	e <sub>2</sub>	PSA-NH-CH <sub>2</sub> -CH <sub>2</sub> - <u>CH<sub>2</sub></u> -C <sub>6</sub> H <sub>5</sub>	2.75	c <sub>2</sub>
PSA-NH-CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> - <u>C<sub>6</sub>H<sub>5</sub>-ORTHO/PARA</u>	7.32	d <sub>2</sub> /f <sub>2</sub>	PSA-NH-CH <sub>2</sub> - <u>CH<sub>2</sub></u> -CH <sub>2</sub> -C <sub>6</sub> H <sub>5</sub>	2.00	b <sub>2</sub>
PSA-NH- <u>CH<sub>2</sub></u> -CH <sub>2</sub> -CH <sub>2</sub> -C <sub>6</sub> H <sub>5</sub>	3.02	a <sub>2</sub>			

**Table S5.** <sup>1</sup>H-NMR peak shifts of PBA-g-PSA in D<sub>2</sub>O.

Group	δ ppm	Letter	Group	δ ppm	Letter
PSA-NH-CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> - <u>C<sub>6</sub>H<sub>5</sub>-META</u>	7.38	f <sub>3</sub>	PSA-NH-CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> - <u>CH<sub>2</sub></u> -C <sub>6</sub> H <sub>5</sub>	2.68	d <sub>3</sub>
PSA-NH-CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> - <u>C<sub>6</sub>H<sub>5</sub>-ORTHO/PARA</u>	7.30	e <sub>3</sub> /g <sub>3</sub>	PSA-NH-CH <sub>2</sub> - <u>CH<sub>2</sub></u> - <u>CH<sub>2</sub></u> -C <sub>6</sub> H <sub>5</sub>	1.70	b <sub>3</sub> /c <sub>3</sub>
PSA-NH- <u>CH<sub>2</sub></u> -CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -C <sub>6</sub> H <sub>5</sub>	3.02	a <sub>3</sub>			

**Table S6.**  $^1\text{H-NMR}$  peak shifts of POE-*g*-PSA in  $\text{D}_2\text{O}$ .

Group	$\delta$ ppm	Letter	Group	$\delta$ ppm	Letter
PSA-NH-CH <sub>2</sub> -CH <sub>2</sub> O- <u>C<sub>6</sub>H<sub>5</sub>-META</u>	7.41	d <sub>4</sub>	PSA-NH-CH <sub>2</sub> - <u>CH<sub>2</sub></u> O- C <sub>6</sub> H <sub>5</sub>	4.30	b <sub>4</sub>
PSA-NH-CH <sub>2</sub> -CH <sub>2</sub> O- <u>C<sub>6</sub>H<sub>5</sub>-ORTHO</u>	7.10	c <sub>4</sub>	PSA-NH- <u>CH<sub>2</sub></u> -CH <sub>2</sub> O- C <sub>6</sub> H <sub>5</sub>	3.40	a <sub>4</sub>
PSA-NH-CH <sub>2</sub> -CH <sub>2</sub> O- <u>C<sub>6</sub>H<sub>5</sub>-PARA</u>	7.06	e <sub>4</sub>			

**Table S7.**  $^1\text{H-NMR}$  peak shifts of 33DPP-*g*-PSA in  $\text{D}_2\text{O}$ .

Group	$\delta$ ppm	Letter	Group	$\delta$ ppm	Letter
PSA-NH-CH <sub>2</sub> -CH <sub>2</sub> -CH <u>C<sub>6</sub></u> <u>H<sub>5</sub>-META</u> C <sub>6</sub> H <sub>5</sub> - <u>META</u>	7.41	e <sub>5</sub>	PSA-NH- <u>CH<sub>2</sub></u> -CH <sub>2</sub> -CHC <sub>6</sub> H <sub>5</sub> C <sub>6</sub> H <sub>5</sub>	2.95	a <sub>5</sub>
PSA-NH-CH <sub>2</sub> -CH <sub>2</sub> -CH <u>C<sub>6</sub></u> <u>H<sub>5</sub>-ORTHO/PARA</u> C <sub>6</sub> H <sub>5</sub> - <u>ORTHO/PARA</u>	7.29	d <sub>5</sub> /f <sub>5</sub>	PSA-NH-CH <sub>2</sub> - <u>CH<sub>2</sub></u> -CHC <sub>6</sub> H <sub>5</sub> C <sub>6</sub> H <sub>5</sub>	2.48	b <sub>5</sub>
PSA-NH-CH <sub>2</sub> -CH <sub>2</sub> - <u>CHC<sub>6</sub></u> H <sub>5</sub> C <sub>6</sub> H <sub>5</sub>	4.12	c <sub>5</sub>			

**Table S8.** LD<sub>50</sub> Values of PTAG-*g*-PSA micelles towards LBC3 GMB cell type are proportional to PTAG alkyl chain length and composition. LD<sub>50</sub> values will decrease as increased hydrophobicity is added to the PTAG group either by longer chain length or added grafting (DOS) as a result of interaction with the cell membrane.

PTAG- <i>g</i> -PSA	LD <sub>50</sub> ( $\mu\text{g/mL}$ )		
	20–30%	50–60%	90–100%
PEA- <i>g</i> -PSA	1033 $\pm$ 99	751 $\pm$ 2	640 $\pm$ 7
PPA- <i>g</i> -PSA	763 $\pm$ 26	556 $\pm$ 30	576 $\pm$ 44
PBA- <i>g</i> -PSA	702 $\pm$ 78	699 $\pm$ 14	418 $\pm$ 155
POE- <i>g</i> -PSA	>1000	>1000	>1000
33DPP- <i>g</i> -PSA	278 $\pm$ 34	118 $\pm$ 2	-

**Table S9.** LC/EC of DOX Loaded POE-*g*-PSA and 33DPP-*g*-PSA Micelles with Varying DOS. Differences between formulations of different DOS were compared for both POE-*g*-PSA and 33DPP-*g*-PSA formulations through a one-way ANOVA with a post-hoc Tukey HSD test.

POE- <i>g</i> -PSA			33DPP- <i>g</i> -PSA		
Group	DOS	p-value	Group	DOS	p-value
P <sub>1</sub>	20–30% vs. 50–60%	n.s.	D <sub>1</sub>	20–30% vs. 50–60%	<0.0001
P <sub>2</sub>	20–30% vs. 90–100%	<0.01	D <sub>2</sub>	20–30% vs. 90–100%	<0.0001
P <sub>3</sub>	50–60% vs. 90–100%	<0.05	D <sub>3</sub>	50–60% vs. 90–100%	<0.0001

**Table S10.** LC/EC of POE-g-PSA and 33DPP-g-PSA Micelles at Varying DOX Feed Rates (90–100% DOS). Differences between the same formulation though at different DOX feed rates was compared through a one-way ANOVA with a post-hoc Tukey HSD test.

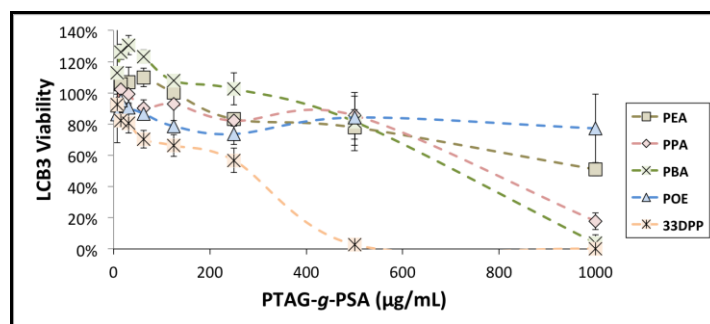
POE-g-PSA			33DPP-g-PSA		
Group	DOX <sub>FEED</sub>	p-value	Group	DOX <sub>FEED</sub>	p-value
P <sub>1</sub>	5% vs. 10%	<0.05	D <sub>1</sub>	5% vs. 10%	<0.01
P <sub>2</sub>	5% vs. 15%	n.s.	D <sub>2</sub>	5% vs. 15%	n.s.
P <sub>3</sub>	10% vs. 15%	n.s.	D <sub>3</sub>	10% vs. 15%	<0.01

**Table S11.** Sizes of POE-g-PSA and 33DPP-g-PSA Micelles at Varying DOX Feed Rates (90–100% DOS). Differences between the same formulation though at different DOX feed rates was compared through a one-way ANOVA with a post-hoc Tukey HSD test. 33DPP D<sub>0</sub>; D<sub>5</sub>; D<sub>10</sub>; D<sub>15</sub>: post-hoc Tukey HSD test not performed based upon ANOVA results.

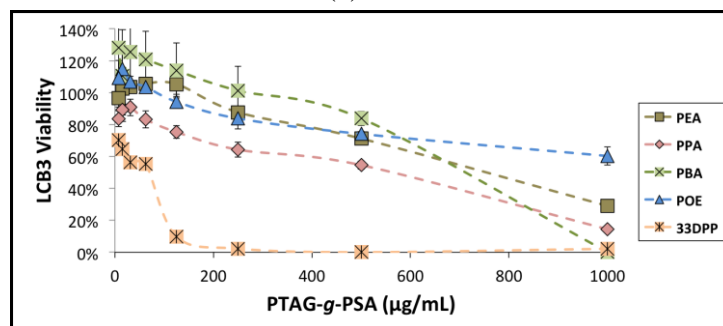
POE-g-PSA					
Group	DOX <sub>FEED</sub>	p-value	Group	DOX <sub>FEED</sub>	p-value
P <sub>0</sub>	0% vs. 5%	<0.01	P <sub>10</sub>	10% vs. 15%	<0.01
P <sub>0</sub>	0% vs. 10%	<0.01	P <sub>15</sub>	15% vs. 0%	<0.01
P <sub>5</sub>	5% vs. 10%	<0.01			

**Table S12.** Stability of POE-g-PSA and 33DPP-g-PSA Micelles at Varying DOX Feed Rates (90–100% DOS). Differences between the same formulation though at different DOX feed rates was compared through a one-way ANOVA with a post-hoc Tukey HSD test.

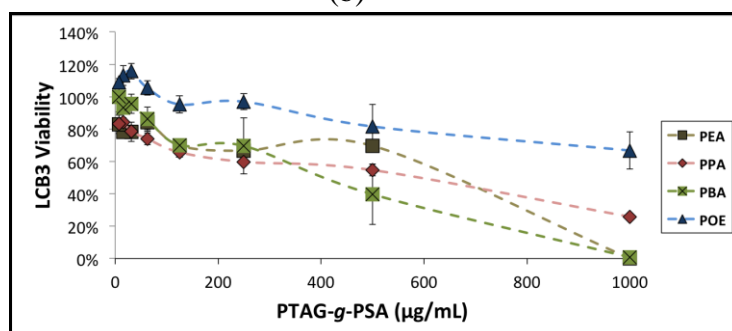
POE-g-PSA			33DPP-g-PSA		
Group	DOX <sub>FEED</sub>	p-value	Group	DOX <sub>FEED</sub>	p-value
P <sub>0</sub>	0% vs. 5%	n.s.	D <sub>0</sub>	0% vs. 5%	n.s.
P <sub>0</sub>	0% vs. 10%	<0.01	D <sub>0</sub>	0% vs. 10%	n.s.
P <sub>5</sub>	5% vs. 10%	<0.01	D <sub>5</sub>	5% vs. 10%	n.s.
P <sub>10</sub>	10% vs. 15%	<0.01	D <sub>10</sub>	10% vs. 15%	<0.01
P <sub>15</sub>	15% vs. 0%	<0.01	D <sub>15</sub>	15% vs. 0%	<0.01



(a)

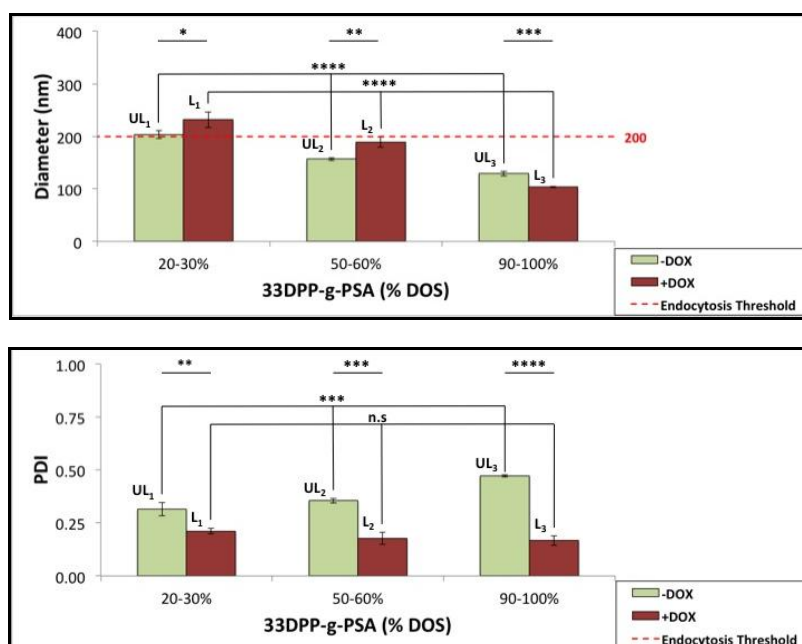


(b)



(c)

**Figure S1.** Cytotoxicity of PTAG-g-PSA micelles towards LBC3 GMB cell type is highly dependent upon PTAG composition and DOS. Cell toxicity increases with higher DOS of each PTAG group; (a) 20–30%; (b) 50–60%; (c) 90–100%. Decreasing the alkyl chain length or enhancing the polarity of the PTAG group effectively decreases the cytotoxic response of LBC3 cells.

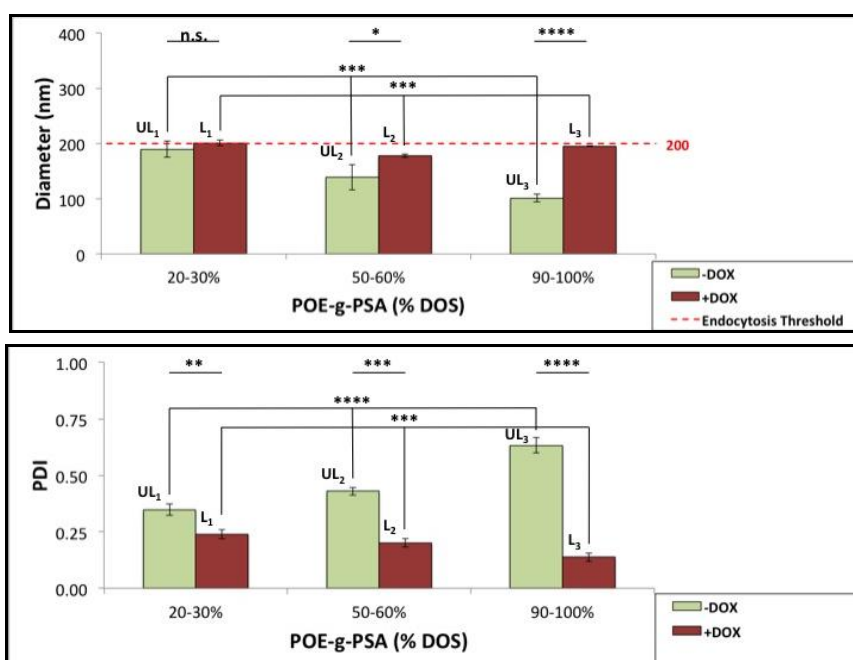


**Figure S2.** Size and stability of 33DPP-g-PSA/DOX Micelles with Varying DOS. Variation in the DOS (20–30%, 50–60%, 90–100%) of 33DPP-g-PSA results in tailorable sizes of both unloaded and DOX-loaded micelles. Stability was greatly increased as a result of DOX loading within the micelle core.

Differences between loaded and unloaded samples at each DOS were statistically analyzed by an F-Test ( $p < 0.05$ ) followed by a Student's  $t$  test assuming equal or unequal variance. \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; \*\*\*\*  $p < 0.0001$ ; n.s. no significance. Differences between formulations of different DOS were compared for both unloaded and DOX-loaded formulations through a one-way ANOVA with a post-hoc Tukey HSD test.

Size:  $L_1$ : 20–30% (DOX) vs. 50–60% (DOX)  $p < 0.01$ ;  $L_2$ : 20–30% (DOX) vs. 90–100% (DOX)  $p < 0.01$ ;  $L_3$ : 50–60% (DOX) vs. 90–100% (DOX)  $p < 0.01$ ;  $UL_1$ : 20–30% vs. 50–60%  $p < 0.01$ ;  $UL_2$ : 20–30% vs. 90–100%  $p < 0.01$ ;  $UL_3$ : 50–60% vs. 90–100%  $p < 0.01$ . Data presentation as mean  $\pm$  SD ( $n = 3$ ).

PDI:  $L_1$ : 20–30% (DOX) vs. 50–60% (DOX) n.s.;  $L_2$ : 20–30% (DOX) vs. 90–100% (DOX) n.s.;  $L_3$ : 50–60% (DOX) vs. 90–100% (DOX) n.s.;  $UL_1$ : 20–30% vs. 50–60% n.s.;  $UL_2$ : 20–30% vs. 90–100%  $p < 0.01$ ;  $UL_3$ : 50–60% vs. 90–100%  $p < 0.01$ . Data presentation as mean  $\pm$  SD ( $n = 3$ ).

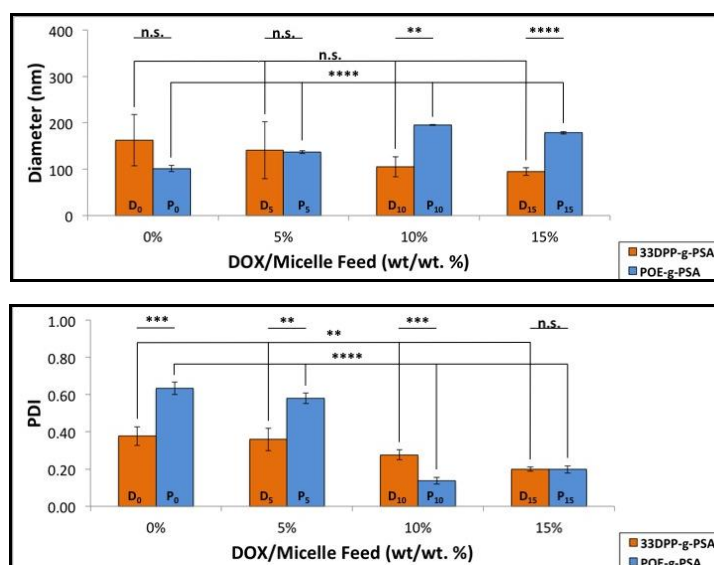


**Figure S3.** Size and stability of POE-g-PSA/DOX Micelles with Varying DOS. Variation in the DOS (20–30%, 50–60%, 90–100%) of POE-g-PSA results in tailorable sizes of both unloaded and DOX-loaded micelles. Stability was greatly increased as a result of DOX loading within the micelle core.

Differences between loaded and unloaded samples at each DOS were statistically analyzed by an F-Test ( $p < 0.05$ ) followed by a student's  $t$  test assuming equal or unequal variance. \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; \*\*\*\*  $p < 0.0001$ ; n.s. no significance. Differences between formulations of different DOS were compared for both unloaded and DOX-loaded formulations through a one-way ANOVA with a post-hoc Tukey HSD test.

Size:  $L_1$ : 20–30% (DOX) vs. 50–60% (DOX)  $p < 0.01$ ;  $L_2$ : 20–30% (DOX) vs. 90–100% (DOX) n.s.;  $L_3$ : 50–60% (DOX) vs. 90–100% (DOX)  $p < 0.01$ ;  $UL_1$ : 20–30% vs. 50–60%  $p < 0.05$ ;  $UL_2$ : 20–30% vs. 90–100%  $p < 0.01$ ;  $UL_3$ : 50–60% vs. 90–100% n.s.

PDI:  $L_1$ : 20–30% (DOX) vs. 50–60% (DOX) n.s.;  $L_2$ : 20–30% (DOX) vs. 90–100% (DOX)  $p < 0.01$ ;  $L_3$ : 50–60% (DOX) vs. 90–100% (DOX)  $p < 0.05$ ;  $UL_1$ : 20–30% vs. 50–60%  $p < 0.05$ ;  $UL_2$ : 20–30% vs. 90–100%  $p < 0.01$ ;  $UL_3$ : 50–60% vs. 90–100%  $p < 0.01$ .



**Figure S4.** Sizes of POE-g-PSA and 33DPP-g-PSA Micelles at Varying DOX Feed Rates (90–100% DOS). Micelles form at different sizes based upon the composition of the core. 33DPP-g-PSA formulations already contain sufficient hydrophobicity can form smaller micelles at higher feed rates due to earlier micelle formation. POE-g-PSA micelles are more polar within their cores, allowing for more DOX to be entrapped on a per micelle basis and leading to larger sizes upon DOX encapsulation based upon later micelle formation. The stabilizing of the micelle core is initiated only at a feed rate greater than 10% DOX. POE micelles stabilize greater than 33DPP formulations at 10% while both reach saturation at 15%.

Differences between POE-g-PSA and 33DPP-g-PSA micelles at each feed rate were statistically analyzed by an F-Test ( $p < 0.05$ ) followed by a student's  $t$  test assuming equal or unequal variance. \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; \*\*\*\*  $p < 0.0001$ ; n.s. no significance. Differences between the same formulation though at different DOX feed rates was compared through a one-way ANOVA with a post-hoc Tukey HSD test.

Size: POE  $P_0$ : 0% vs. 5% (DOX)  $p < 0.01$ ;  $P_0$ : 0% vs. 10% (DOX)  $p < 0.01$ ;  $P_5$ : 5% vs. 10% (DOX)  $p < 0.01$ ;  $P_{10}$ : 10% vs. 15% (DOX)  $p < 0.01$ ;  $P_{15}$ : 15% vs. 0% (DOX)  $p < 0.01$ . 33DPP  $D_0$ ;  $D_5$ ;  $D_{10}$ ;  $D_{15}$ : Post-hoc Tukey HSD test not performed based upon ANOVA results. Data presentation as mean  $\pm$  SD ( $n = 3$ ).

PDI: POE  $P_0$ : 0% vs. 5% (DOX) n.s.;  $P_0$ : 0% vs. 10% (DOX)  $p < 0.01$ ;  $P_5$ : 5% vs. 10% (DOX)  $p < 0.01$ ;  $P_{10}$ : 10% vs. 15% (DOX)  $p < 0.01$ ;  $P_{15}$ : 15% vs. 0% (DOX)  $p < 0.01$ . 33DPP  $D_0$ : 0% vs. 5% (DOX) n.s.;  $D_0$ : 0% vs. 10% (DOX) n.s.;  $P_5$ : 5% vs. 10% (DOX) n.s.;  $P_{10}$ : 10% vs. 15% (DOX)  $p < 0.01$ ;  $P_{15}$ : 15% vs. 0% (DOX)  $p < 0.01$ . Data presentation as mean  $\pm$  SD ( $n = 3$ ).

