SUPPLEMENTAL INFORMATION

As shown in Table 1, a panel of sixteen NPCs were synthesized via reversible addition fragmentation chain transfer (RAFT) polymerizations and characterized for polymer composition and physical characteristics, including molecular weight, corona-to-core molecular weight ratio, hydrophobic core composition, and size, for use in this study. While the raw polymer characterization data is presented in Table 1, graphical representations of the NPC diversity evaluated for use with the saturated farnesol formulation are shown in Figure S1. This panel was designed to leverage the knowledge gained from previous studies, which indicated that NPCs with high CCR and small coronas and diameters enhance drug bioavailability within biofilms [28, 29]. However, we also wanted to examine how hydrophobic core composition affected NPC efficacy for polymers aligned with these previous design criteria as well as for multiple polymers with molecular weights, sizes, or CCRs spanning a range of values.

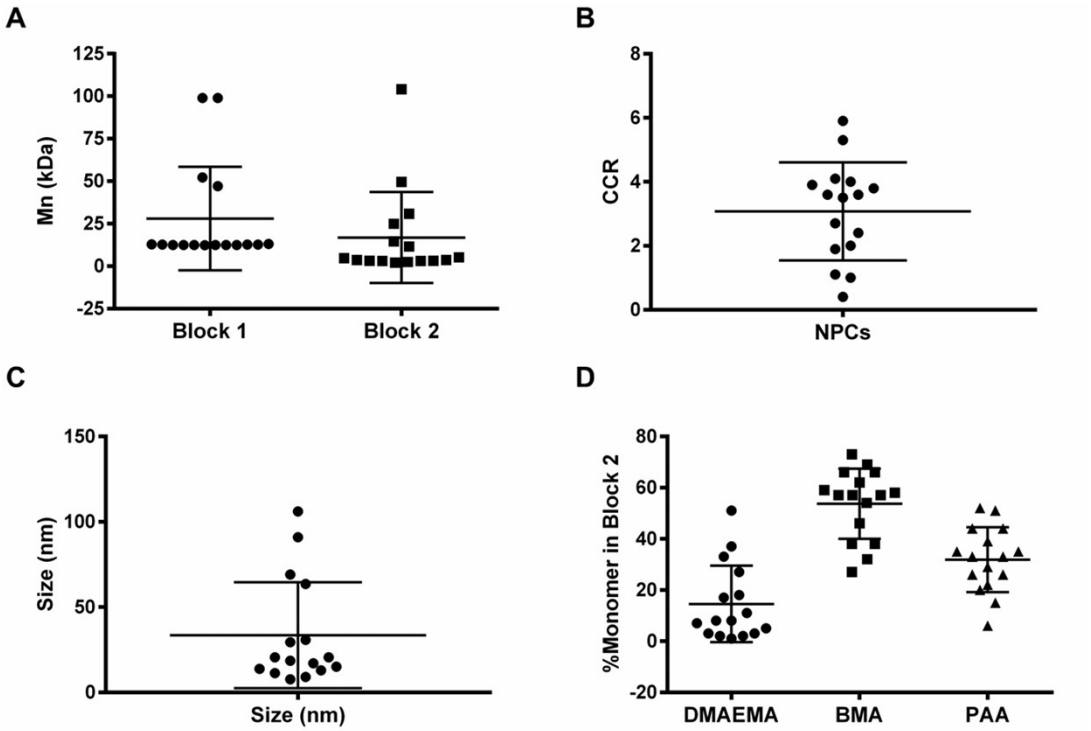

Figure S1. Graphical summary of the diversity of NPCs evaluated in this study. A) Block 1 and Block 2 molecular weights for the polymers used in this study. B) Corona-to-core molecular weight ratios (CCR) of the polymers used in this study. C) Size (diameter in nm) of the NPCs used in this study. D) Percent monomer composition values in the hydrophobic core (Block 2) of the polymers used in this study estimated from ^{1}H NMR analysis. Data presented as individual dot plots showing mean and standard deviation.

Critical micelle concentration (CMC) testing confirmed that the saturated farnesol-loaded NPC formulation improved NPC stability regardless of CCR (Supplemental Figure S2).

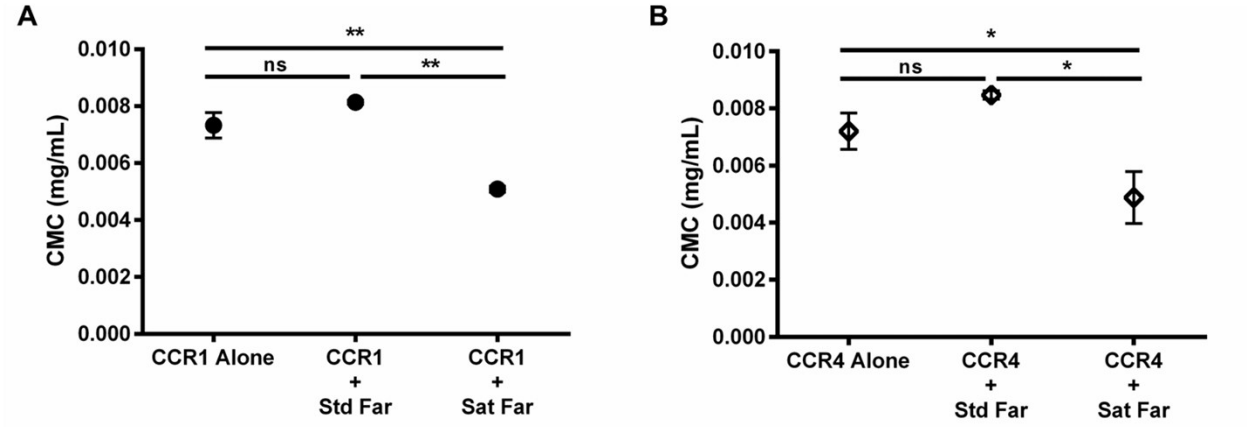

Figure S2. Saturated farnesol-loaded NPC formulation improved NPC stability. A) CCR 1 (black, filled circles) and B) CCR 4 (white, open diamonds) critical micelle concentration (CMC) results for NPC alone, standard loaded NPCs, and saturated loaded NPCs. Data shown as average and standard deviation from n=2-4 independent experiments. * $p \leq 0.05$, ** $p \leq 0.01$, and ns = no significant difference from One-way ANOVA with Tukey's multiple comparisons test. Abbreviations: CCR, Corona-to-Core molecular weight Ratio; Far, farnesol; NPC, nanoparticle carrier; Sat, saturated farnesol formulation; Std, standard farnesol formulation.

To initially assess how the NPCs shown in Table 1 and Figure S1 performed in terms of pH-responsive antibacterial effectiveness against *S. mutans*, minimum inhibitory concentration (MIC) tests were performed for each NPC unloaded or loaded with saturated farnesol conditions at pH 5 and pH 7 using planktonic *S. mutans* UA159. These results indicate that the farnesol MIC values for NPCs loaded with saturated drug conditions are generally lower at pH 5 than at pH 7 (Supplemental Table S1), which represents a pH-responsive trend in antibacterial effectiveness against planktonic *S. mutans*. The prominent farnesol MIC against planktonic *S. mutans* at pH 5 for this formulation (16 μg/mL) was at least a 2-fold reduction compared to that of pH 7 and the farnesol alone in phosphate buffer MIC values at either pH 5 or pH 7 (128 μg/mL) from Supplemental Table S1. This MIC value at pH 5 was also aligned with the reported MIC value for farnesol dissolved in ethanol prior to assay execution regardless of pH as shown in Table S1 and reported previously [49]. Moreover, only NPCs with CCR values > 2 and diameters < 29 nm showed this 2-fold MIC reduction. No difference in MIC value between pH 5 and pH

7 was observed for formulations containing NPCs with CCR values ≤ 2 and diameters ≥ 29 nm. In addition, the MIC and MCBK values for all the unloaded NPCs tested were > 128 μg/mL, which was the highest concentration used for unloaded NPC samples in this study.

Table S1. Farnesol and NPC MIC and MCBK values for saturated formulation using *S. mutans* in planktonic and biofilm state

Polymer	Farnesol Concentration (μg/mL)				NPC Concentration (μg/mL)			
	Planktonic (MIC)		16h Biofilm (MCBK)		Planktonic (MIC)		16h Biofilm (MCBK)	
	pH 7	pH 5	pH 7	pH 5	pH 7	pH 5	pH 7	pH 5
NP12/2	32	16	>128	128	>64	>64	>128	>128
NP12/3a	16	16	>128	128	>64	>64	>128	>128
NP12/3b	32	16	>128	128	>64	>64	>128	>128
NP12/3c	16	16	>128	128	>64	>64	>128	>128
NP12/4	16	16	>128	128	>64	>64	>128	>128
NP12/5	32	32	>128	128	>64	>64	>128	>128
NP12/12	16	16	>128	128	>64	>64	>128	>128
NP13/2	32	16	>128	128	>64	>64	>128	>128
NP13/3	32	16	>128	>128	>64	>64	>128	>128
NP13/4	32	16	>128	128	>64	>64	>128	>128
NP13/5	32	16	>128	>128	>64	>64	>128	>128
NP13/31	32	32	>128	128	>64	>64	>128	>128
NP47/25	32	32	>128	128	>64	>64	>128	>128
NP52/15	32	16	>128	>128	>64	>64	>128	>128
NP99/50	32	32	>128	128	>64	>64	>128	>128
NP99/104	32	32	>128	128	>64	>64	>128	>128
Farnesol (in PB)	128	128	>128	>128	N/A	N/A	N/A	N/A
Farnesol (in EtOH/PB)	16	16	N/A	N/A	N/A	N/A	N/A	N/A

Abbreviations: EtOH, ethanol; MCBK, minimum concentration biofilm killing; MIC, minimum inhibitory concentration; PB, phosphate buffer.

To quantify the antibacterial effectiveness using colony forming units (CFUs) per mL of the saturated formulation conditions with the panel of NPCs on *S. mutans*, samples from each of the MIC tests in Supplemental Table S1 were plated on agar using serial dilutions and the CFUs were counted as illustrated in Supplemental Figure S3 below.

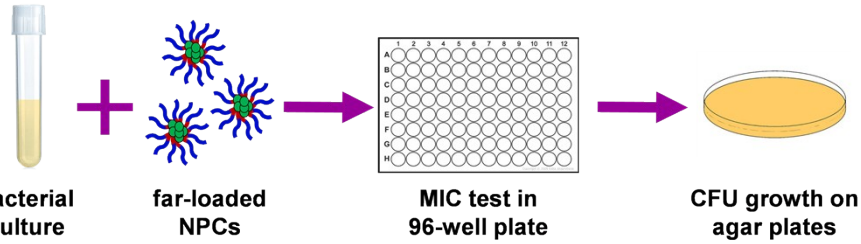

Figure S3. Illustration summarizing the MIC testing process and the CFU growth assay used to evaluate the antibacterial effectiveness of the saturated farnesol-loaded NPC formulation against planktonic *S. mutans*. *S. mutans* UA159 serotype c cells were grown in ultra-filtered tryptone-yeast extract (UFTYE) broth containing 1% glucose (w/v) at 37 °C and 5% CO_2 to mid-exponential phase. Farnesol-loaded NPCs were prepared in UFTYE broth at pH 7.0 and 5.0, and dispensed into a 96-well plate (90 µl per well). Ten µl of *S. mutans* suspension (10^6 colony forming units (CFU)/ml) was added to each well, and the MIC was determined as the lowest concentration of the test agent that inhibited CFU growth on agar plates after a 24 h incubation at 37 °C with 5% CO_2.

Results from this analysis are shown in Figure 4 and discussed in the main text. In addition, the CFU/mL results for all of the unloaded NPCs are shown in Figure S4 and demonstrated no differences in the CFU/mL values for any of the NPCs at pH 5 or pH 7 compared to the PBS control group.

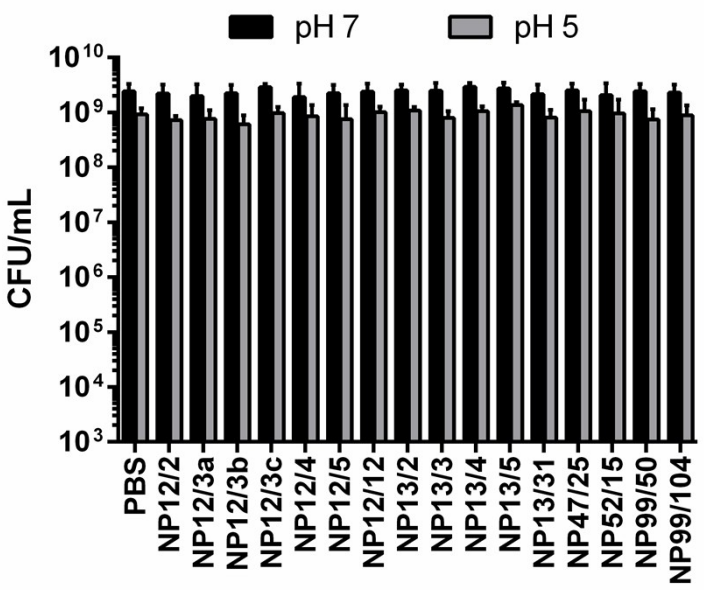

Figure S4. Effectiveness of unloaded NPCs (16 µg/mL NPC) against planktonic *S. mutans* at pH 5 and pH 7 as determined by CFU/mL. Each NPC from Table 1 and Table S1 was evaluated for its antibacterial effectiveness against planktonic *S. mutans* at pH 5 (grey bars) and pH 7 (black bars) using the process shown in Figure S3.

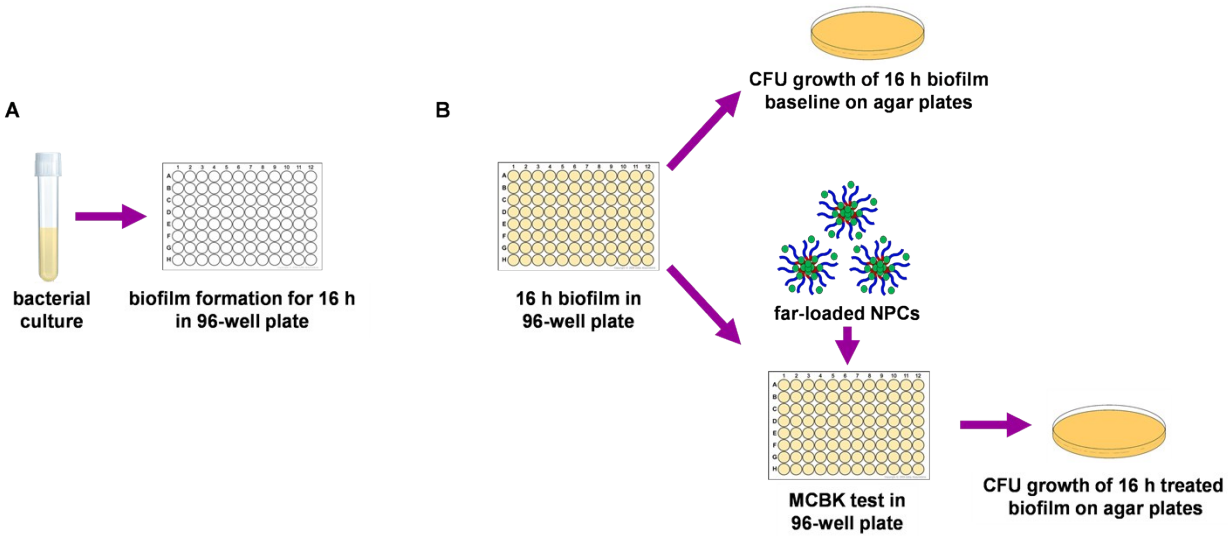

Figure S5. Illustration summarizing the MCBK testing process used to evaluate antibacterial effectiveness of the saturated farnesol-loaded NPC formulation against 16-hour *S. mutans* biofilms. A) The MCBK plate was inoculated with ~2 × 10^5 CFU of *S. mutans* per ml in each well and then was incubated for 16 h. B) Serial dilutions of farnesol-loaded nanoparticles were prepared and added to the preformed biofilms on the MCBK plate after washing the plate. The CFU of 16 h biofilm and 16 h treated biofilm were counted and compared to determine the MCBK.

Similarly, minimum concentration for biofilm killing (MCBK) tests using 16-hour *S. mutans* biofilms were conducted as described in the Experimental section of the main text and as shown in Supplemental Figure S5. The results from the MCBK analysis for the saturated farnesol-NPC formulations are shown in Figure 4 and discussed in the main text. In addition, the CFU/mL results for all of the unloaded NPCs screened against 16-hour *S. mutans* biofilms are shown in Figure S6 and demonstrated no differences in the CFU/mL values for any of the NPCs at pH 5 or pH 7 compared to the PBS control group.

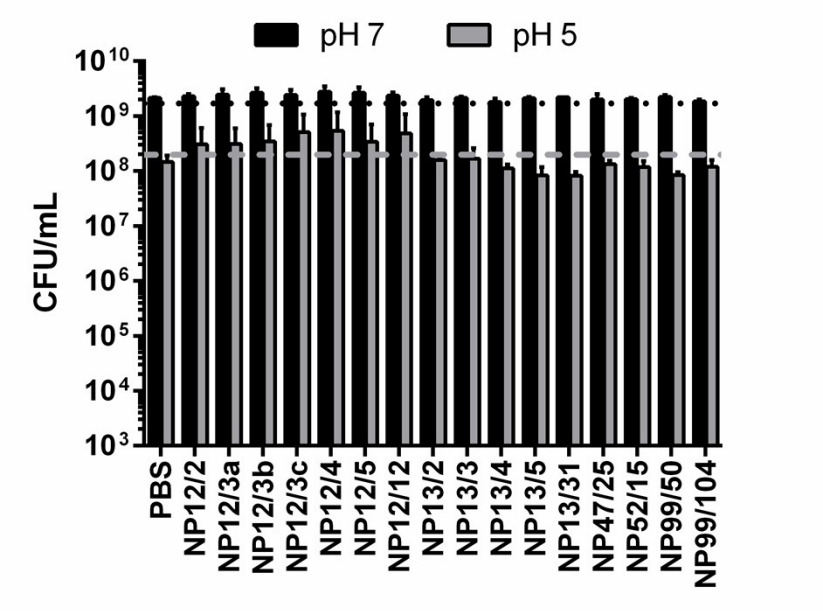

Figure S6. Effectiveness of unloaded NPCs (128 µg/mL NPC) against 16-hour *S. mutans* biofilms at pH 5 and pH 7 as determined by CFU/mL. Each NPC from Table 1 and Table S1 was evaluated for its antibacterial effectiveness against 16-hour *S. mutans* biofilms at pH 5 (grey bars) and pH 7 (black bars) using the process shown in Figure S5. Black dotted line and grey dashed line represent average CFU/mL values for unloaded NPC formulations at pH 7 (~$1.7x10^9$ CFU/mL) and pH 5 (~$2.0x10^8$ CFU/mL), respectively.

As part of the mechanistic investigations of farnesol-loaded NPC interactions with *S. mutans*, zeta potential analyses of the standard and saturated farnesol-loaded NPCs formulations compared to the NPC alone were conducted. Interestingly, these analyses revealed no significant difference in NPC surface charge regardless of the formulation approach used (see Supplemental Figure S7 below).

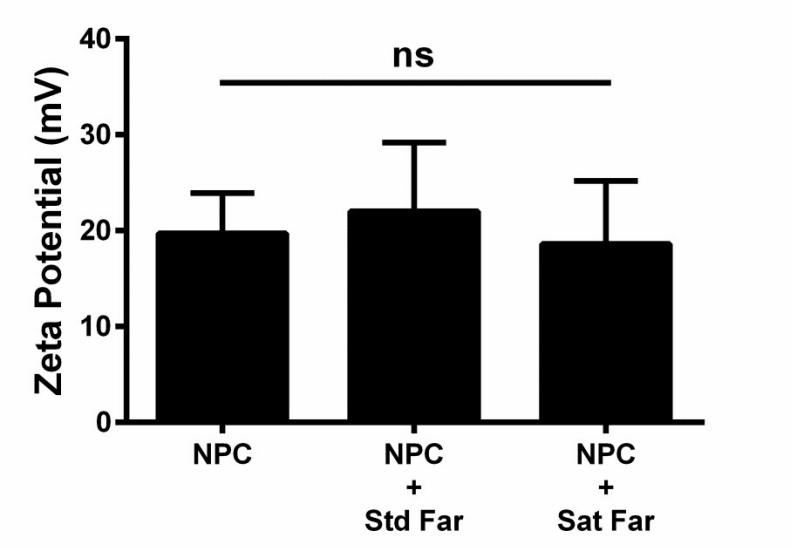

Figure S7. Zeta potential analyses of the standard and saturated farnesol-loaded NPCs formulations compared to unloaded NPC. Zeta potential results showing no statistically significant difference in surface charge among NPC alone (*i.e.*, NP13/4), standard farnesol-loaded NPC, and saturated farnesol-loaded NPC formulations. Data shown as average and standard deviation from n=5 zeta measurements per group. ns = no significant difference from One-way ANOVA with Tukey's multiple comparisons test.

The versatility of the saturated drug-NPC formulation was tested using thonzonium bromide. Similar to the farnesol-containing formulations, minimum inhibitory concentration (MIC) and minimum concentration for biofilm killing (MCBK) tests using planktonic and 16-hour biofilm *S. mutans*, respectively, were performed for NPCs with CCR 1 and CCR 4 (NP12/12 and NP12/3c) loaded with saturated thonzonium bromide conditions at pH 5 and pH 7. The MIC and MCBK results from this testing is shown in Table S2.

Table S2. Thonzonium bromide and NPC MIC values for saturated formulation using planktonic *S. mutans*

Polymer	Formulation	Thonzonium Bromide Concentration (μg/mL)		NPC Concentration (μg/mL)	
		Planktonic (MIC)		Planktonic (MIC)	
		pH 7	pH 5	pH 7	pH 5
NP12/12	Standard	1.25	1.25	8.44	8.44
	Saturated	1.25	1.25	1.56	1.56
NP12/3c	Standard	1.25	1.25	8.44	8.44
	Saturated	1.25	1.25	1.56	1.56
TB Alone	N/A	2.5	1.25	N/A	N/A

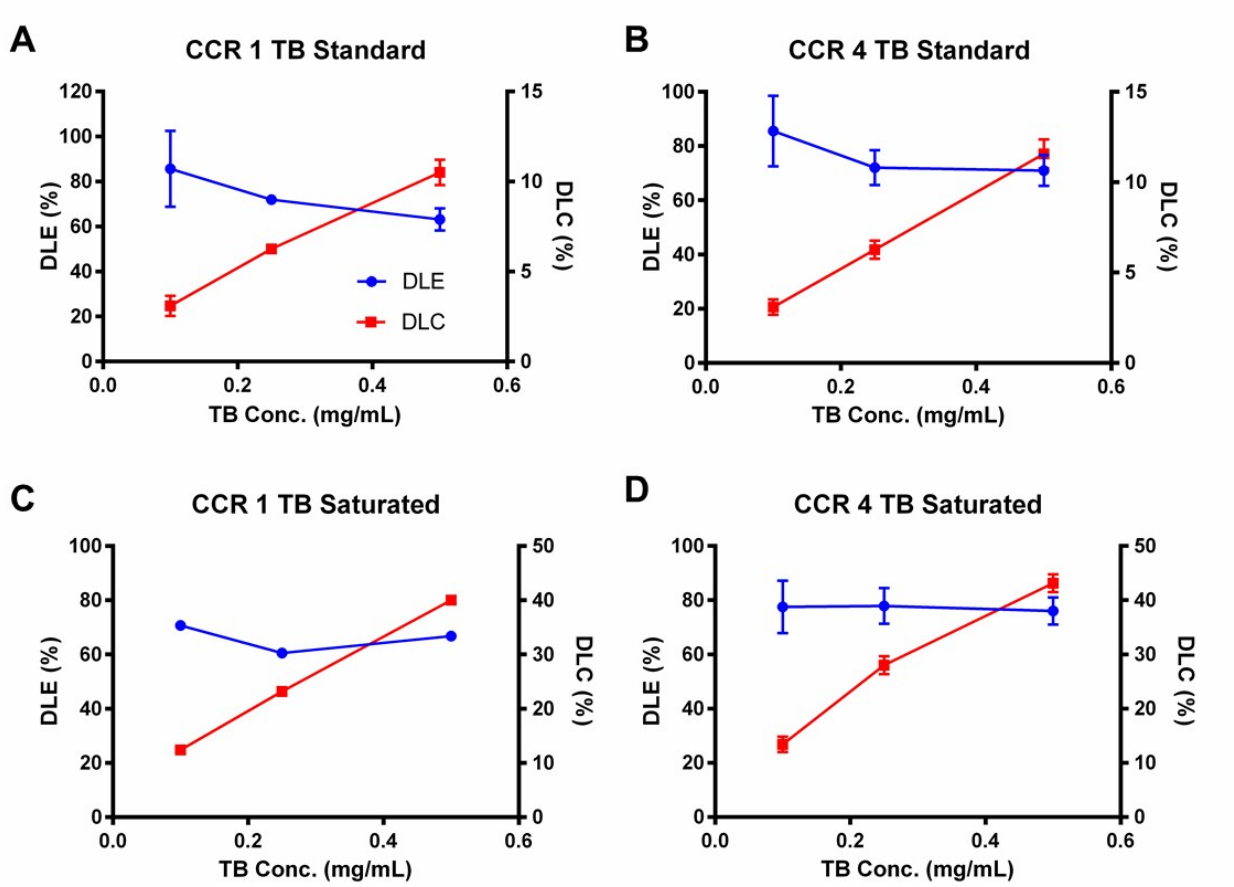

Figure S8. Thonzonium bromide drug loading efficiency and drug loading capacity curves for standard and saturated drug formulations. A & B) Drug loading efficiency (DLE) and drug loading capacity (DLC) curves for standard thonzonium bromide formulations using CCR 1 (A) and CCR 4 (B). C & D) DLE and DLC curves for saturated thonzonium bromide formulations using CCR 1 (C) and CCR 4 (D). Data shown as mean and standard deviation from n = 2-4 independent experiments.

For each sample shipment from Rochester, NY to Philadelphia, PA, two frozen gel packs were removed from -80 °C at least 3 hours prior to the planned shipment drop-off time and stored in a closed standard laboratory foam shipping container at room temperature. This step enabled sufficient pre-packout temperature conditioning for the container and warmed the gel packs prior to sample packing to prevent sample freezing. Samples consisting of lyophilized polymer and/or aqueous solutions containing NPC and/or drugs in 20 mL scintillation vials were either wrapped in protective dunnage (*e.g.*, packing peanuts, paper towels, and/or aluminum foil) or loaded in cardboard boxes. These protected sample containers were placed in the foam shipping container between the two frozen gel packs and covered in additional protective dunnage to maintain interior container temperatures between 0 and 8 °C for at least

24 hours during overnight shipment. This level of temperature control maintained sample stability (*e.g.*, no detectable physical changes; data not shown). Test shipments between labs confirmed reproducible temperature control throughout the duration of a representative overnight shipment (Supplemental Figure S9). Upon shipment receipt, aqueous samples were stored at 2-8 °C and lyophilized polymer samples were stored at room temperature until use.

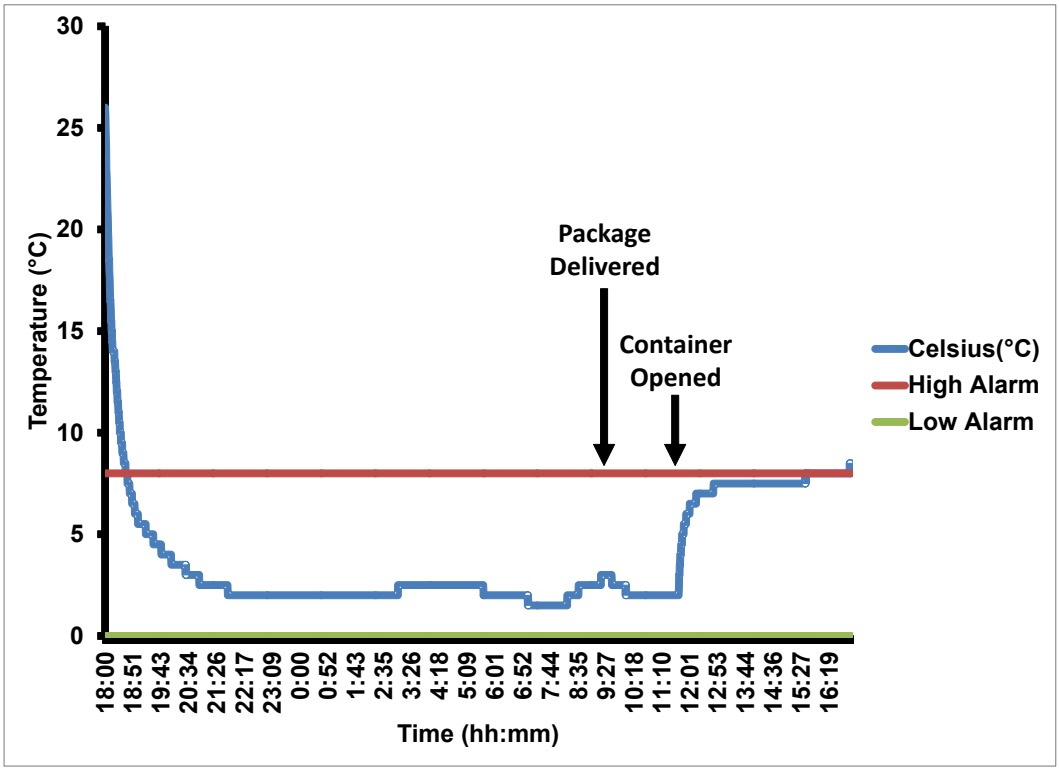

Figure S9. Test shipment temperature profile for package sent from Rochester, NY to Philadelphia, PA. Package interior temperature profile for a representative test shipment between Rochester, NY and Philadelphia, PA confirmed robust, reproducible temperature control throughout the duration of a representative overnight shipment between the Benoit and Koo labs.