Supporting Information

Polymer Synthesis

Poly(D, L-lactide-co-glycolide)-*b*-poly(L-histidine)-*b*-poly(ethylene glycol) (PLGA-PLH-PEG) was synthesized using a sequential end-grafting process. First, the poly(L-histidine)-*b*-poly(ethylene glycol) (PLH-PEG) diblock copolymer was formed as demonstrated in "Rxn A" of Scheme S1. PLH is a 22 or 32-mer peptide synthesized and purified by HPLC by GenScript (Piscataway, NJ) with the following sequence, from N- to C-terminus: Lys-$(His)_{20}$-Cys or Lys-$(His)_{30}$-Cys. 0.0128 mmol of this reactant was dissolved in 1 mL water and pH adjusted to pH 6.5 using 0.5 M NaOH dropwise. 0.0282 mmol of mPEG-OPSS polymer obtained from Laysan Bio (MW 5000, Arab, AL) was dissolved in 3 mL DMSO. The two reactants were mixed together at room temperature for 24 hours to yield a yellowish white solution of the PLH-PEG diblock copolymer (product **1**, Scheme S1). The product was purified by dialyzing against pure water using 2,000 MWCO Slide-A-Lyzer G2 dialysis membranes (Thermo Scientific, Billerica, MA) followed by lyophilization for 48 hours. 0.0222 mmol of PLGA-COOH (inherent viscosity 0.67, LACTEL, Cupertino, CA) was dissolved in 2 mL dichloromethane and reacted with 0.9160 mmol 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 1.1025 mmol of N-hydroxysuccinimide (NHS) for 4 hours at room temperature while stirring (Rxn B, Scheme S1). The resulting activated PLGA-NHS ester was precipitated twice in ~ -20°C anhydrous methanol. The PLGA-NHS ester was dried *in vacuo* for 2 hours then dissolved in 3 mL of DMSO. 10 umol of PLGA-NHS in DMSO were reacted with 14.6 umol of PLH-PEG dissolved in 1 mL of DMSO. To this reaction mixture, 115 umol of N,N-diisopropylethylamine (DIEA) were added and the reaction was allowed to proceed overnight at room temperature. The resulting crude triblock copolymer product was precipitated in a ~ -20°C 50/50 v/v mixture of methanol/diethyl ether, redissolved in DMSO, then precipitated again to produce a white polymer product. This product was dried *in vacuo* for at least 4 hours. The final purified product **2** was collected as a yellowish white brittle solid at a mass yield varying between 50-

99%. The reaction products and intermediates were characterized using MALDI-TOF, ^{1}H-NMR, and gel permeation chromatography (GPC). PLGA-PEG was synthesized similarly using the same starting materials and excess reactants, with the key difference being that the PLGA-NHS was reacted with mPEG-NH_2 (MW 5,000, Laysan Bio, Arab, AL) in dichloromethane.

Polymer Characterization

MALDI-TOF (Voyager-DE STR, JBI Scientific, Huntsville, TX) was used to confirm the conjugation of the PLH and PEG blocks using sinapinic acid to form the matrix and using a 1-15 kDa acquisition mass range. A typical MALDI-TOF spectrum included PEG Mz = 5,315; PLH Mz 2,867; PEG-PLH Mz = 8,169. ^{1}H-NMR (400 MHz, Bruker, Billerica, MA) was used to detect the presence of the different polymer blocks at the various reaction steps. The ^{1}H-NMR spectrum of the final triblock copolymer product PLGA-PLH-PEG demonstrates peaks corresponding to the three different copolymer blocks. Proton shifts in DMSO-d_6 (ppm): 7.6 b (imidazole ring *H*, PLH), 6.8 b (imidazole ring *H*, PLH), 5.2 b (-C*H*- LA unit, PLGA), 4.9 b (-CH_2-, GA unit, PLGA), 4.4 b (α-carbon -C*H*-, PLH), 3.5 s (OCH_2CH_2, PEG), 3.0 b (-CH_2- PLH), 1.5 b (-CH_3 of LA unit, PLGA). GPC was used to measure the molecular weight of the triblock PLGA-PLH-PEG (DMF mobile phase), Mz ~ 88,000 relative to PMMA standards for all PLGA-PLH-PEG polymers.

NP preparation

To form vancomycin-encapsulated NPs, 1 mg of polymer (PLGA-PLH-PEG or PLGA-PEG) dissolved in 15/85 v/v DMSO/ethyl acetate solution was diluted into a final volume of 500 uL of ethyl acetate (with trace DMSO) to form the organic phase. The drug-containing aqueous phase would typically consist of 50 uL of a 4 g/L solution of vancomycin hydrochloride (Sigma Aldrich, St. Louis, MO) dissolved in pure water. The aqueous phase was sonicated into the polymer-containing organic phase for 15 sec at 40% amplitude using a probe tip sonicator (Misonix Sonicator S-4000, Farmingdale, NY).

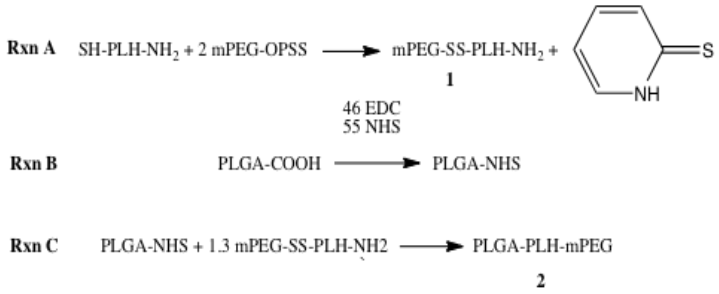
This primary emulsion was then emulsified into 2 mL of a 10% w/v NaCl solution at 40% amplitude for 30 sec. This concentrated double emulsion was diluted into 8 mL of a 5% w/v NaCl solution under magnetic stirring. The NPs were allowed to harden by allowing slow organic solvent evaporation for 4 hours in the hood. NPs were purified by triple filtration using Amicon Ultra-4 100,000 NMWL centrifugal filter units (Millipore, Billerica, MA) using sterile water. To form green fluorescent NP-488s, PLGA-COOH (inherent viscosity 0.67, LACTEL, Cupertino, CA) was coupled to amine-modified Alexa-488 (Life Technologies, Carlsbad, CA) using EDC/NHS chemistry and purified by precipitation and drying *in vacuo*. The resulting Alexa-488-PLGA product was (15% w/w of the total polymer mass) was co-dissolved in the organic phase with the PLGA-PLH-PEG or PLGA-PEG polymer in a final volume of 500 uL. The 500 uL were then sonicated into 2 mL of sterile water at a 40% amplitude setting for 30 seconds. This emulsion was diluted into an additional 8 mL of water, solvent evaporation, then NP-488 purification as described for vancomycin-containing NPs. Fluorescence per mg of NP-488 was not significantly different between triblock and diblock NPs or at different pH, as measured using a plate reader.

Evaluation of degree of PLH polymerization on NP properties and binding to bacteria

To evaluate the relative effects of histidine density on NP binding to bacteria, which might enable tuning for specific biologic applications, we examined the magnitude of impact that an increase in the PLH block degree of polymerization made on pH sensitivity. The results show that increasing the PLH block degree of polymerization while maintaining other parameters constant (constant PLGA and PEG length) results in greater pH sensitivity, with PLGA-PLH-PEG NP zeta potential switching being larger in magnitude (Figure S3A). The surface charge-switching was more rapid and resulted in steeper slope with 30 histidine repeats (PLGA-PLH_{30}-PEG, slope = -13.9 mV/pH unit R^2 = 0.959, N=3) as compared to 20 histidine repeats (PLGA-PLH_{20}-PEG, slope = -8.6 mV/pH unit R^2 = 0.951, N=3) and PLGA-PEG (slope = -2.9 mV/pH unit R^2 = 0.447, N=3). The PLH length did not significantly affect the size of the

NPs at any pH value (Figure S3B). These data suggest that changing the degree of polymerization of the PLH segment is a method by which to modestly increase the degree of pH sensitivity of the PLGA-PLH-PEG NP system. We also evaluated the resulting impact on NP binding to bacteria. These results show that the quantity of PLGA-PLH-PEG NP binding was dependent on the degree of polymerization of the PLH segment ($p < 0.05$, Figure S3C).

Supplemental Figures and Figure Legends



Scheme S1. Synthesis procedure for forming the PLGA-PLH-PEG copolymer. A three step, end-grafting strategy was employed to form the desired PLGA-PLH-PEG **2** copolymer. (Rxn A) Reacting the C-terminal Cys of the PLH block with the orthopyridyldisulfide-modified end group of PEG to form the PLH-PEG **1** diblock copolymer. (Rxn B) Activating the PLGA-COOH using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS). (Rxn C) Conjugating the PLGA-NHS and PEG-PLH polymers using the N-terminal Lys to form the final product **2**.

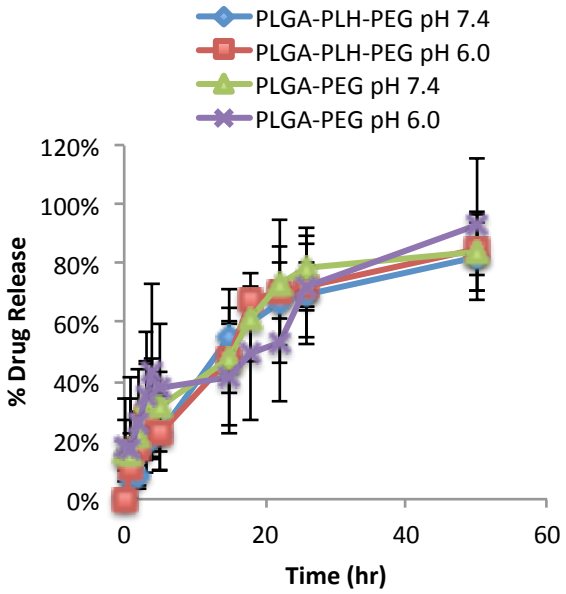


Figure S1. Release of vancomycin from PLGA-PEG and PLGA-PLH-PEG NPs as a function of pH. Drug release curves demonstrate no significant difference in vancomycin release between release curves from PLGA-PEG and PLGA-PLH_{30}-PEG NPs. Drug release study performed in 4 mL pH-adjusted 1x PBS solutions at 37°C.

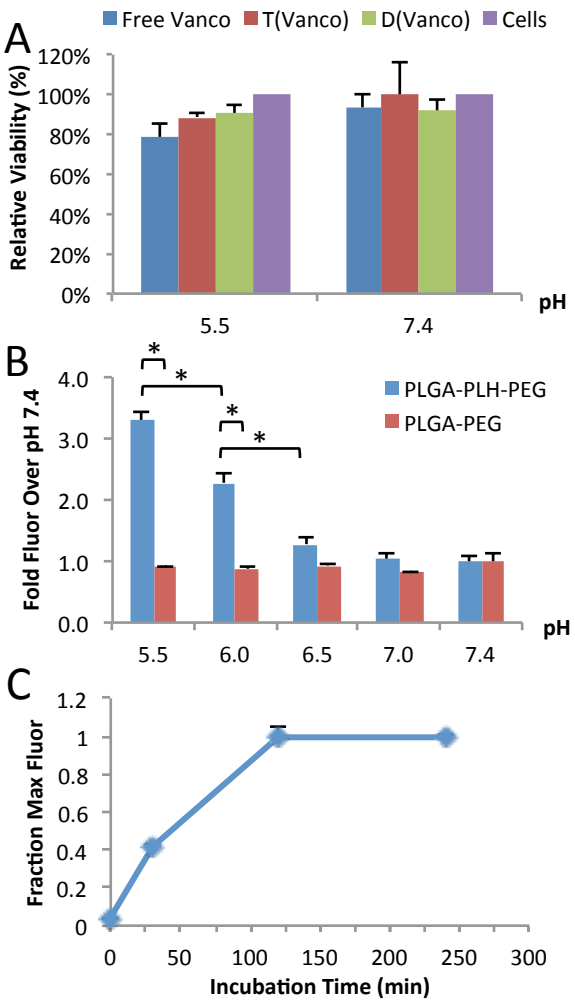


Figure S2: Binding to, and effects of, PLGA-PLH-PEG NPs on human cells. (A) Cell viability assay on a representative human cell line, human prostate adenocarcinoma cells (LNCaP), by alamarBlue (Life Technologies, Carlsbad, CA) at a very high concentration (~35 ug/mL) of vancomycin formulated as: PLGA-PLH-PEG-encapsulated vancomycin (T(Vanco)), PLGA-PEG-encapsulated vancomycin (D(Vanco)) and free vancomycin (Free Vanco) incubated with cells for 1 hour, washed, then assayed for absorbance at 570 nm 48 hours post treatment demonstrates a small reduction at in cell viability that is not significantly different between treated groups (One-way ANOVA, $p > 0.05$, N=3). (B) Binding assay by flow cytometry demonstrates increased binding to LNCaP with decrease in pH. (C) A kinetics of binding assay to human LNCaP cells demonstrates a slower rate of binding to this cell line than to bacteria.

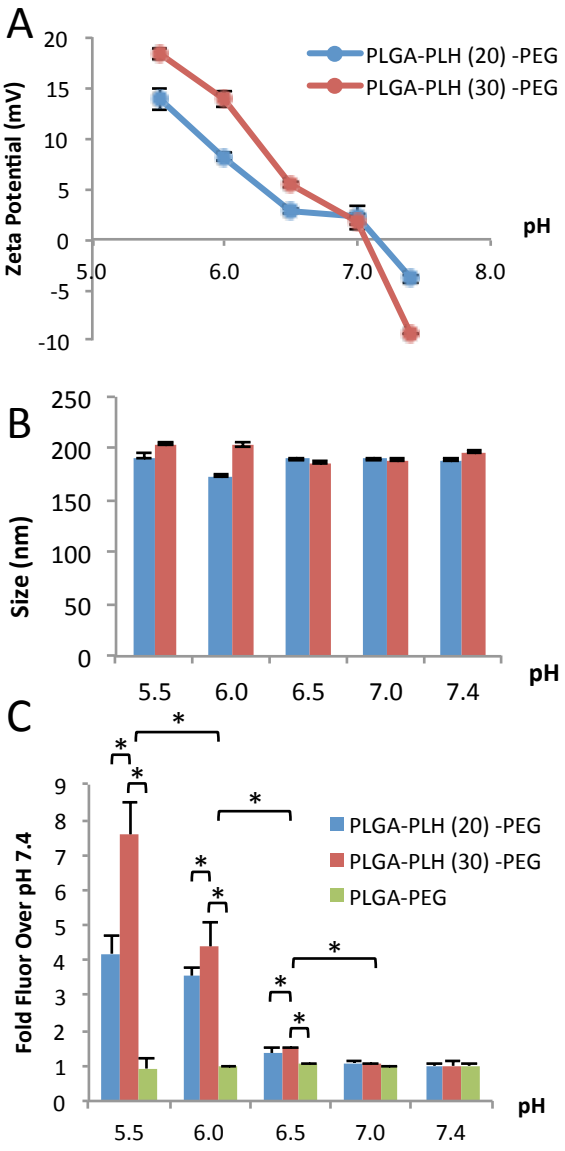


Figure S3. Zeta potential and binding to bacteria as a function of PLH degree of polymerization. (A) NP zeta potential as a function of pH. (B) NP size vs pH. (C) NP binding expressed as fold fluorescence over that observed at pH 7.4 vs pH. Binding to *S. aureus* bacteria increases with decreasing pH and with increasing PLH degree of polymerization, achieving significance at pH $\leq$ 6.5 ($p < 0.05$) (* indicates $p < 0.05$, N=3 for all observations).