Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary Information:

Delivery of enzalutamide via nanoparticles for effectively inhibiting prostate cancer progression

Preparation process and method of monomer and polymer

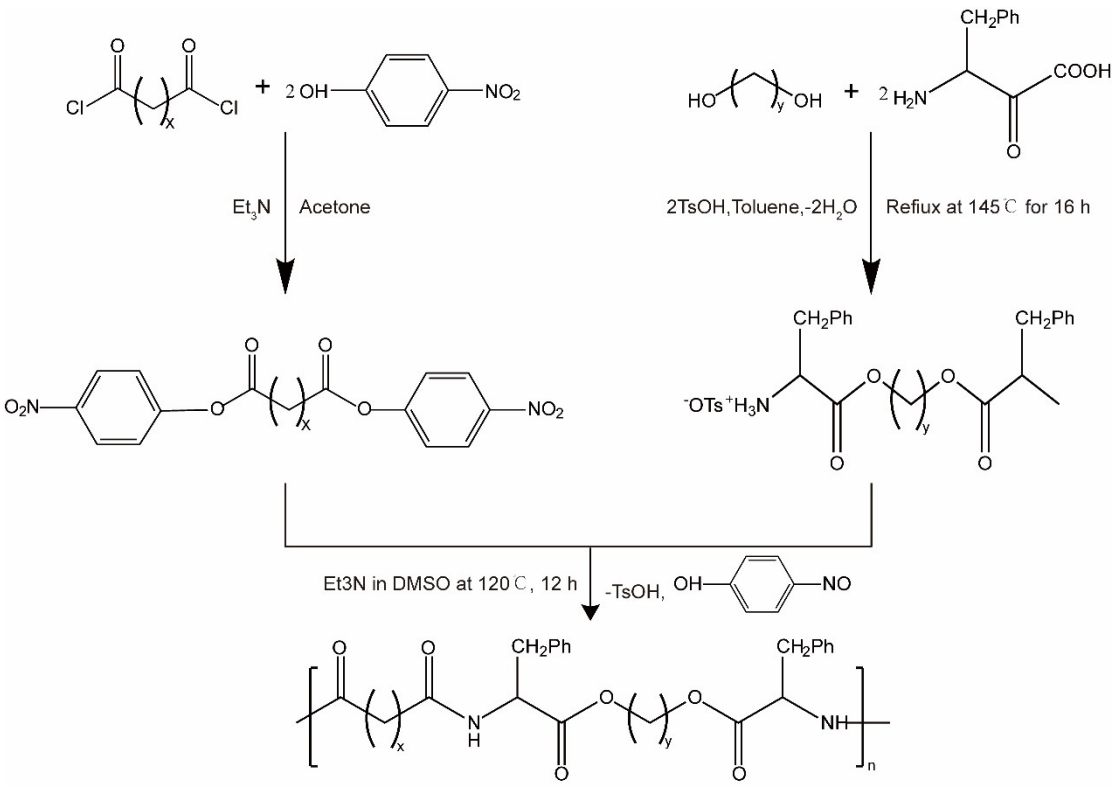
The preparation of the hydrophobic L- phenylalanine-poly (eater amide) (Phe-PEA) polymer mainly consisted of the following parts. First, monomer I was prepared by a condensation reaction to produce dip-nitrophenyl dicarboxylic acid. Then, monomer II was prepared, namely, toluene-4-sulfonate of bis (PHE) alkyl diester was produced by a solid-liquid reaction. The next step was to prepare the Phe-PEA polymer by solution polycondensation (Supplementary Figure 1). The details of the preparation of monomers I and II can be obtained from a previously published article, while the Phe-PEA polymer (yield> 80%) was prepared by optimizing the scheme. Monomers I and II (5 mmol) were added to 8 mL of anhydrous DMSO (8 mL) and then stirred thoroughly after vortexing at 120 ℃. Then, 15 mmol of triethylamine was supplemented. The prepared compound was incubated at 80℃ overnight, and cold ethyl acetate was added for precipitation. The collected sediment was cleaned with methanol and then dried in a vacuum.

The Phe-PEA polymer (x-Phe-y) is synthesized by di-p-nitrophenyl sebacate (N8, x = 8) and toluene-4-sulfonic acid bis (detection) butane diester (Phe-4 y = 4). In the chemical formula, x represents the amount of methylene diacid, and y represents the amount of methylene glycol. The flow rate was 1 ml/min and the solvents were delivered at the ratio of mobile phase A (water): mobile phase B (Acetonitrile) equal to 45: 55, and detection was at 230 nm wavelength, column oven temperature 26°C, with a retention time of 8.72 minutes. The correlation coefficient of the standard curve was

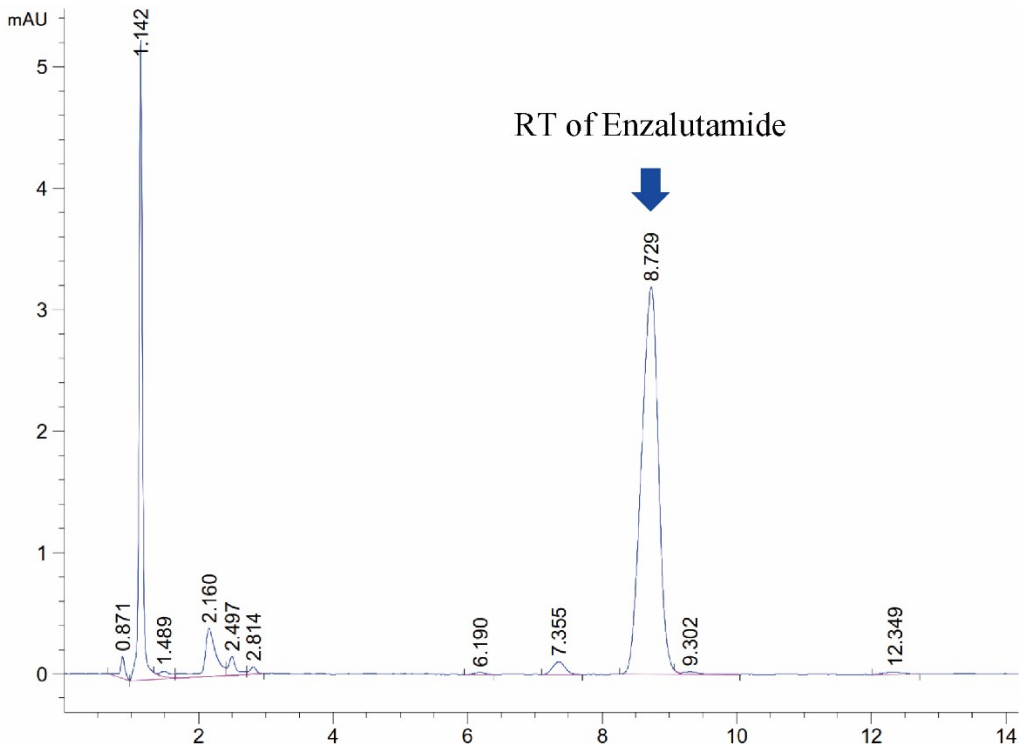
greater than 0.99.

Preparation and Characterization

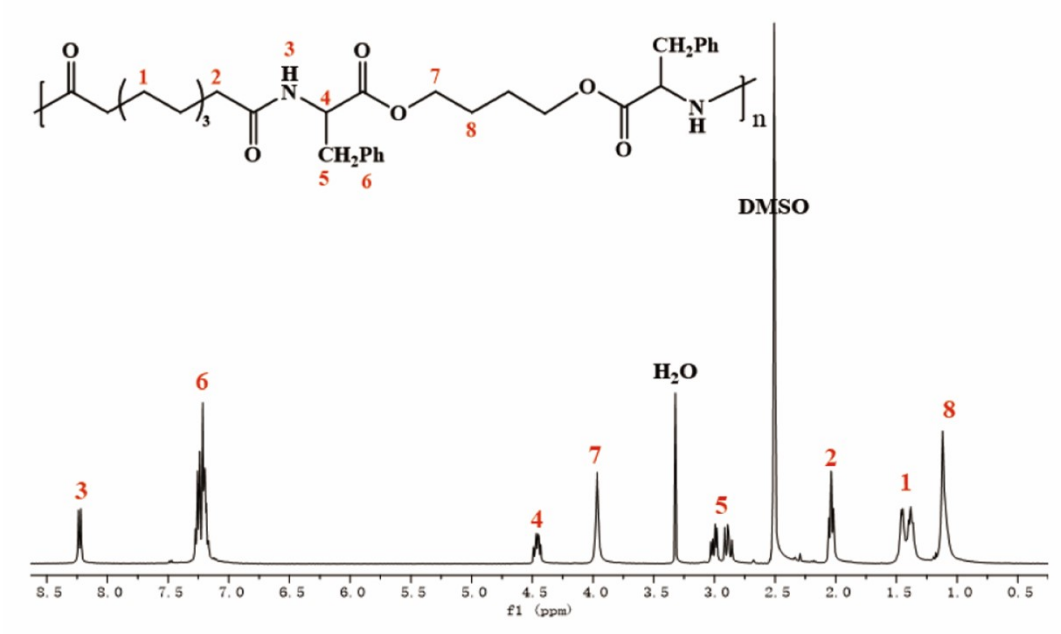
The synthesis method of ENZ-8P4 NPs was obtained by the nano-precipitation method: 10.0 mg of 8-Phe-4 polymer, 40.0 mg DSPE-PEG 2000 and 5.0 mg enzalutamide were soluble in 0.8 mL of dimethyl sulfoxide (DMSO). And then, the compound was dropped into 10.0 mL of water solution and stirred vigorously. The residual free molecules and organic solvents were cleaned twice with PBS and then removed by an Amicon Ultra15 centrifugal filter (MWCO 100 KDa, Millipore, USA). At last, the ENZ-8P4 NPs were dissolved in 1.0 mL PBS for the subsequent experimentation. Blank 8P4 NPs were used as a blank control in subsequent experiments. A predetermined amount of 8P4 polymer and C6(3 wt% NPs) were mixed in dimethyl sulfoxide, and the C6-8P4 NPs were prepared according to the above method. The particle size and zeta potential of the synthesized nanomaterials were measured by dynamic light scattering method (DLS, Zetasizer Nano-ZS90, Malvern, UK). The morphology of the ENZ-8P4 NPs and blank 8P4 NPs were observed and photographed by transmission electron microscopy (TEM, Tecnai G2 Spirit, USA). The carrying capacity of the material was measured by high-performance liquid chromatography (HPLC, Agilent Technologies, USA). The HPLC chromatograms and retention times of Enzalutamide was displayed in supplementary figure 2. The chemical structure of the ENZ-8P4 NPs is shown in supplementary figure 3.



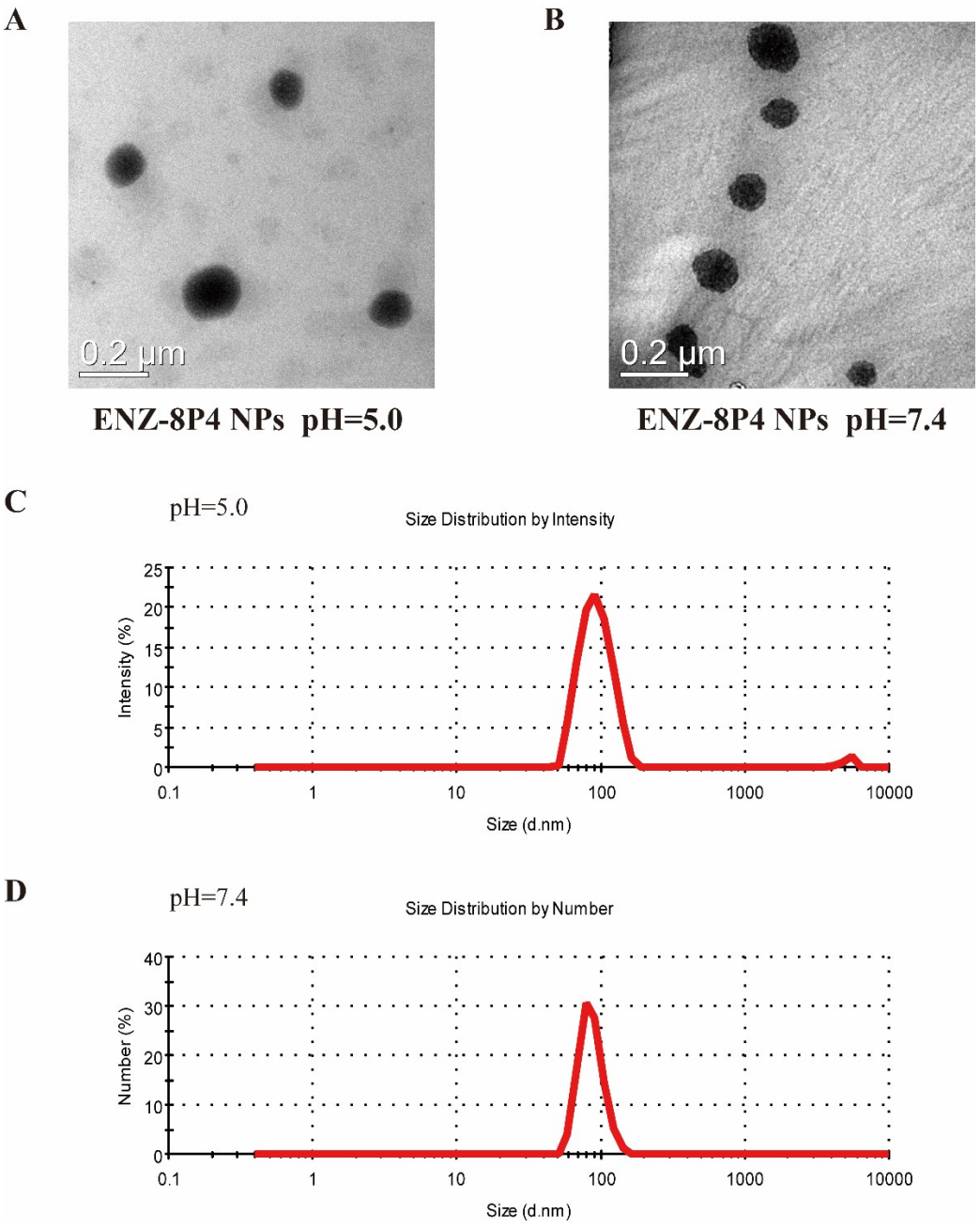
Supplementary Figure 1. Synthetic routes of Phe-PEA polymers.



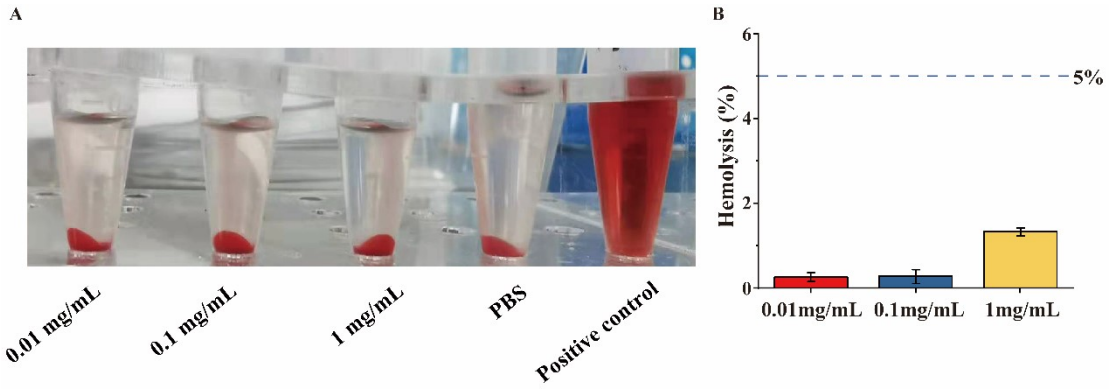
Supplementary Figure 2. HPLC chromatograms and retention times of enzalutamide.



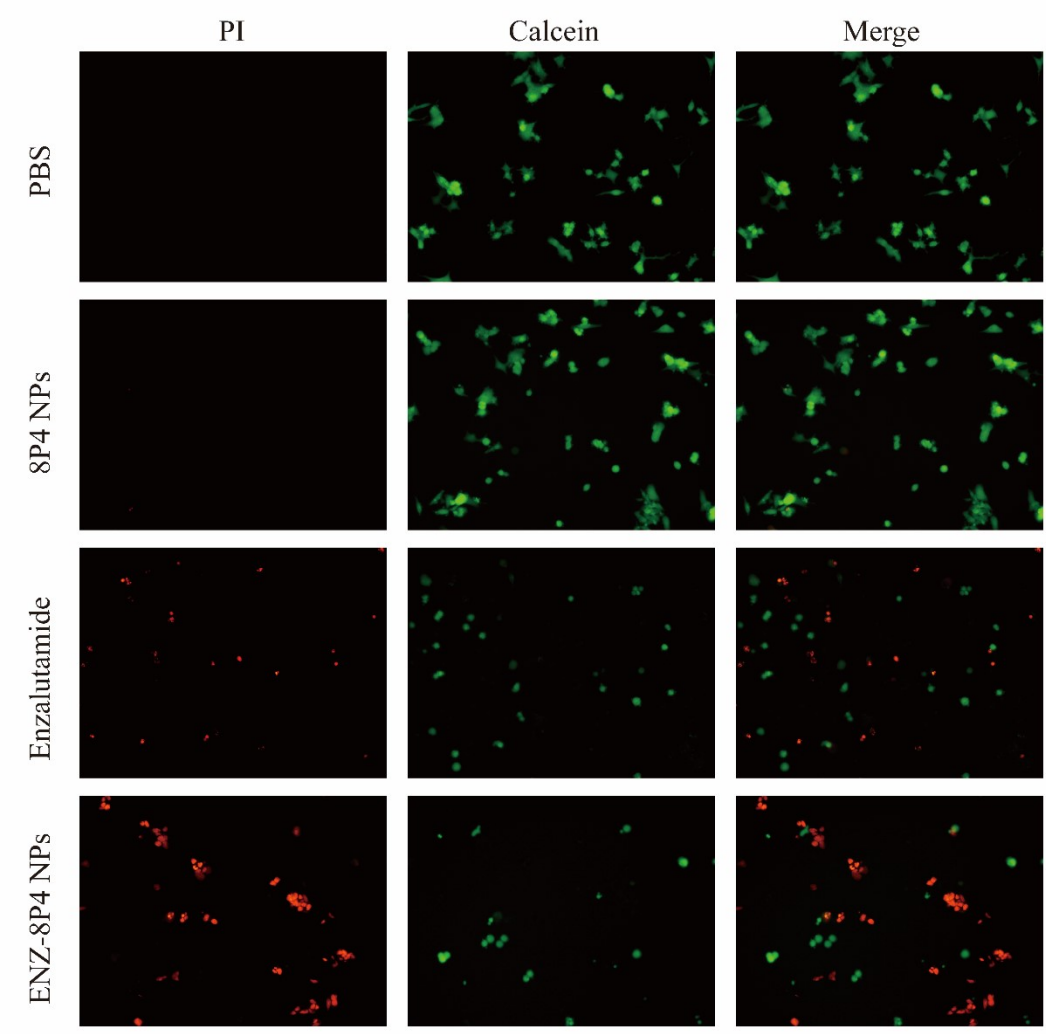
Supplementary Figure 3. The HNMR results depict the successful synthesis of the target compounds.



Supplementary Figure 4. The particle sizes of ENZ-8P4 NPs at different pH values. (A) The morphology of ENZ-8P4 NPs at pH 5.0. (B) The morphology of ENZ-8P4 NPs at pH 7.4. (C-D) The particle size distribution of ENZ-8P4 NPs at different pH values.



Supplementary Figure 5. Hemolysis test.



Supplementary Figure 6. The Living and death cell staining of different treatment groups. Fluorescence images of 22Rv1 cells treated with PBS, 8P4 NPs, ENZ-8P4 NPs and ENZ. Green: Calcein; Red: PI.