Supplementary Information

Denatured Protein Stabilized Drug Nanoparticles: Tunable Drug State and Penetration across the Intestinal Barrier

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Table S1

	Particle size (nm)	PI
Apical medium	157.60	0.269 ± 0.019
Basolateral medium	158.20	0.338 ± 0.023

Table S1. Mean particle size and PI of STP-Ns in apical and basolateral medium after incubation with Transwell filter grown Caco-2 cell monolayer at 37 ^{o}C for 2 h.

Figure S1

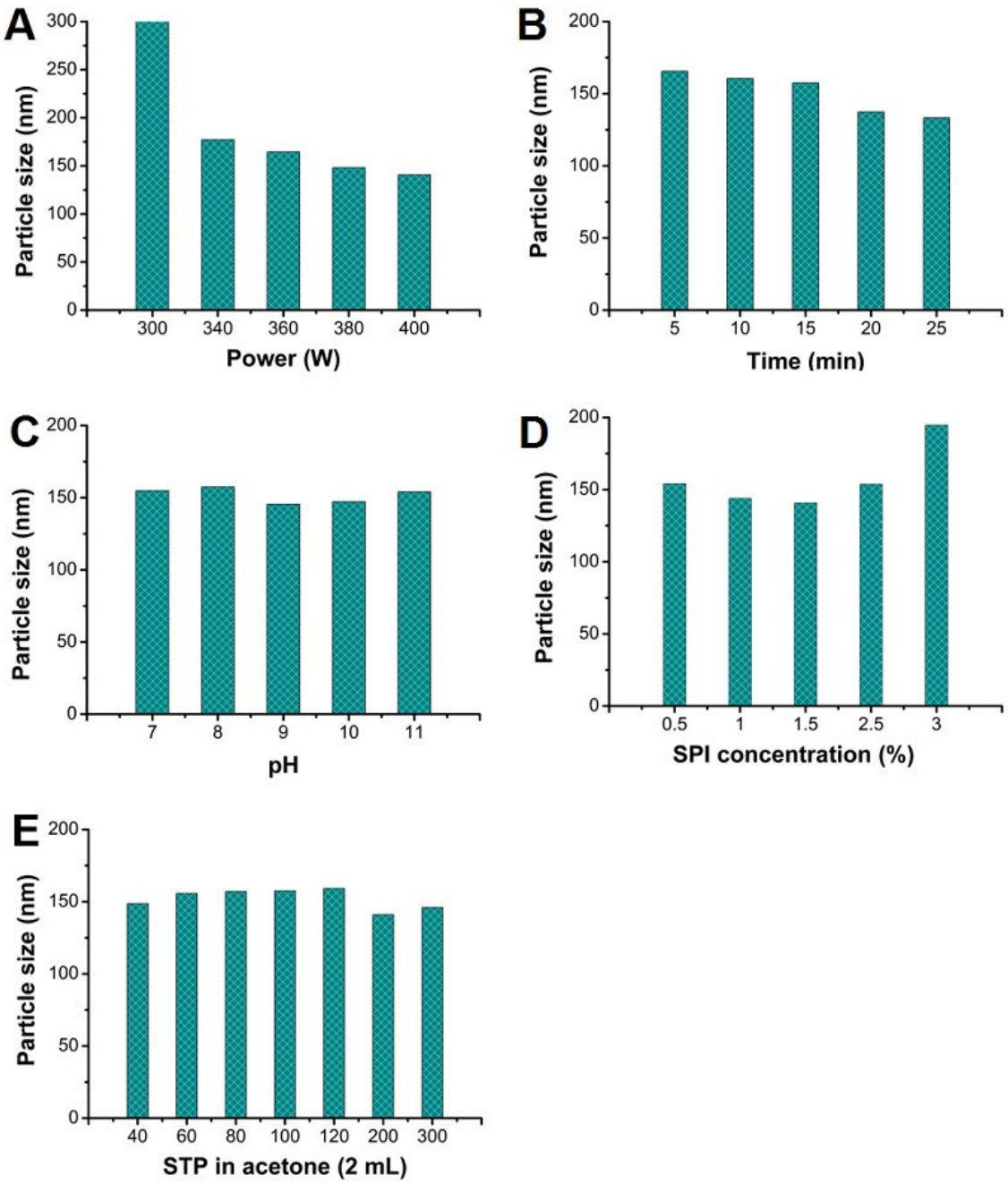

Figure S1. Effects of (**A**) powder input, (**B**) duration of ultrasonic treatment, (**C**) pH, (**D**) SPI concentration and (**E**) STP concentration in acetone on the particle size of the STP-Ns. (**A**, **B**, **C**, **E**) The SPI concentration under aqueous conditions was 1 mg/mL, and (**A**, **B**, **C**, **D**) the STP concentration was 80 mg/mL in 2 mL of acetone.

Figure S2

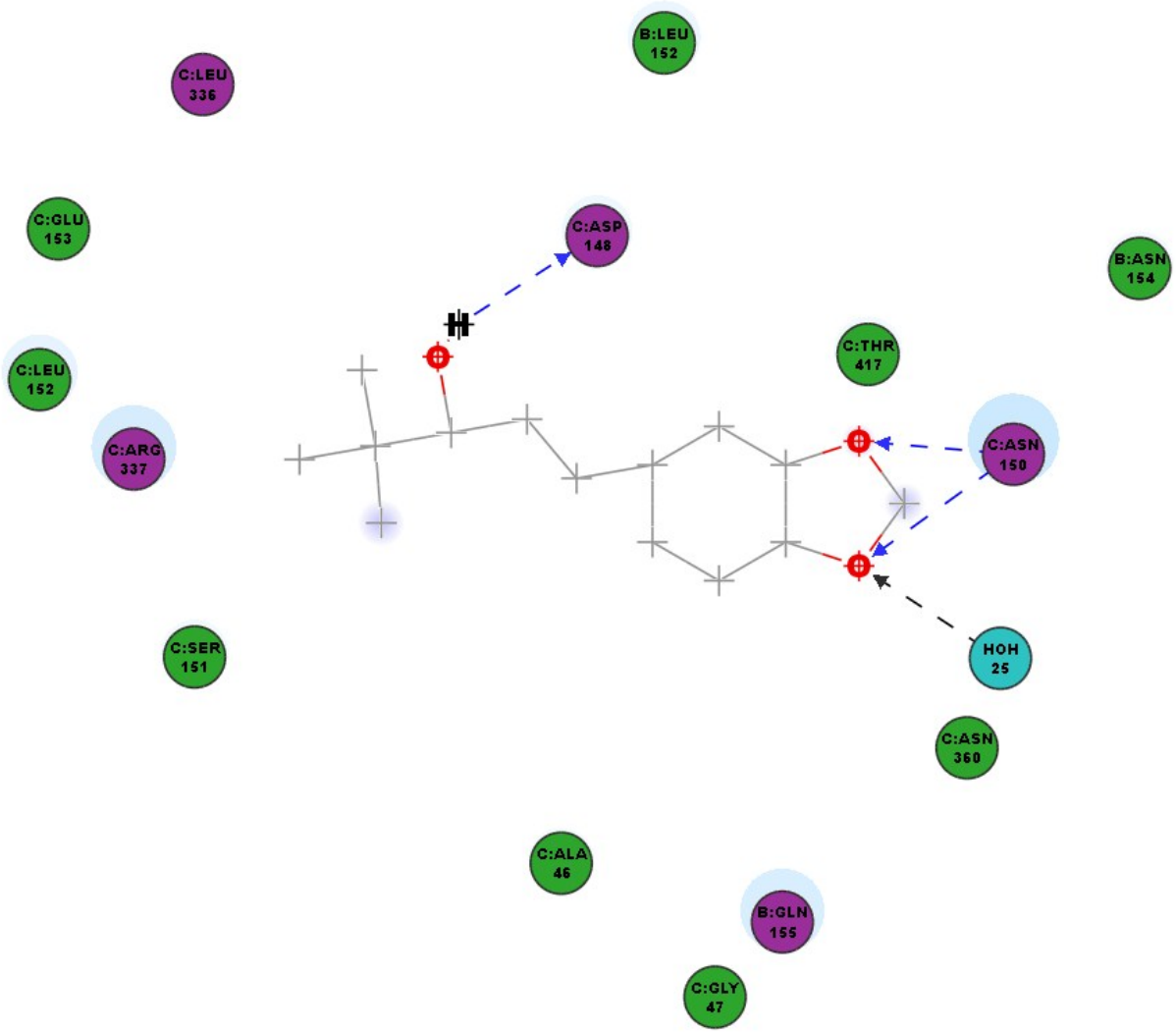

Figure S2. 2D structure of protein-drug complex

Figure S3

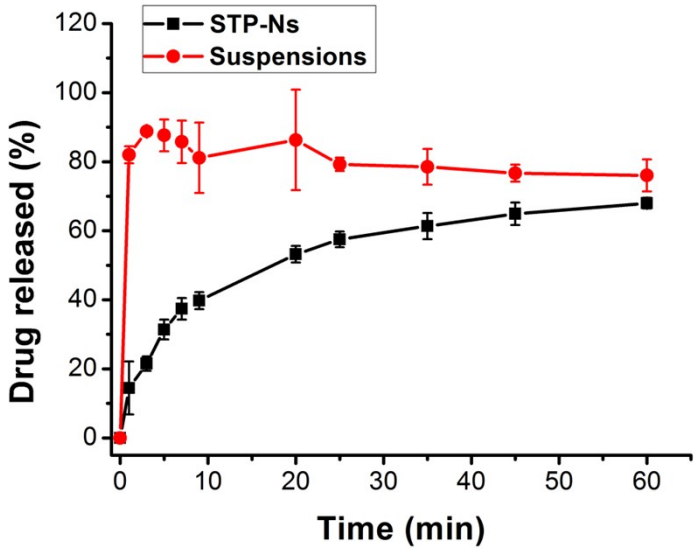

Figure S3. In vitro dissolution profiles of STP from the STP-Ns and suspensions in pH 6.8 PBS containing 1% Tween-80 at 37 °C (n = 3). The control is suspension formulation preparing by dispersing STP raw particles into 2.5% (w/w) HPMC solution.

Figure S4

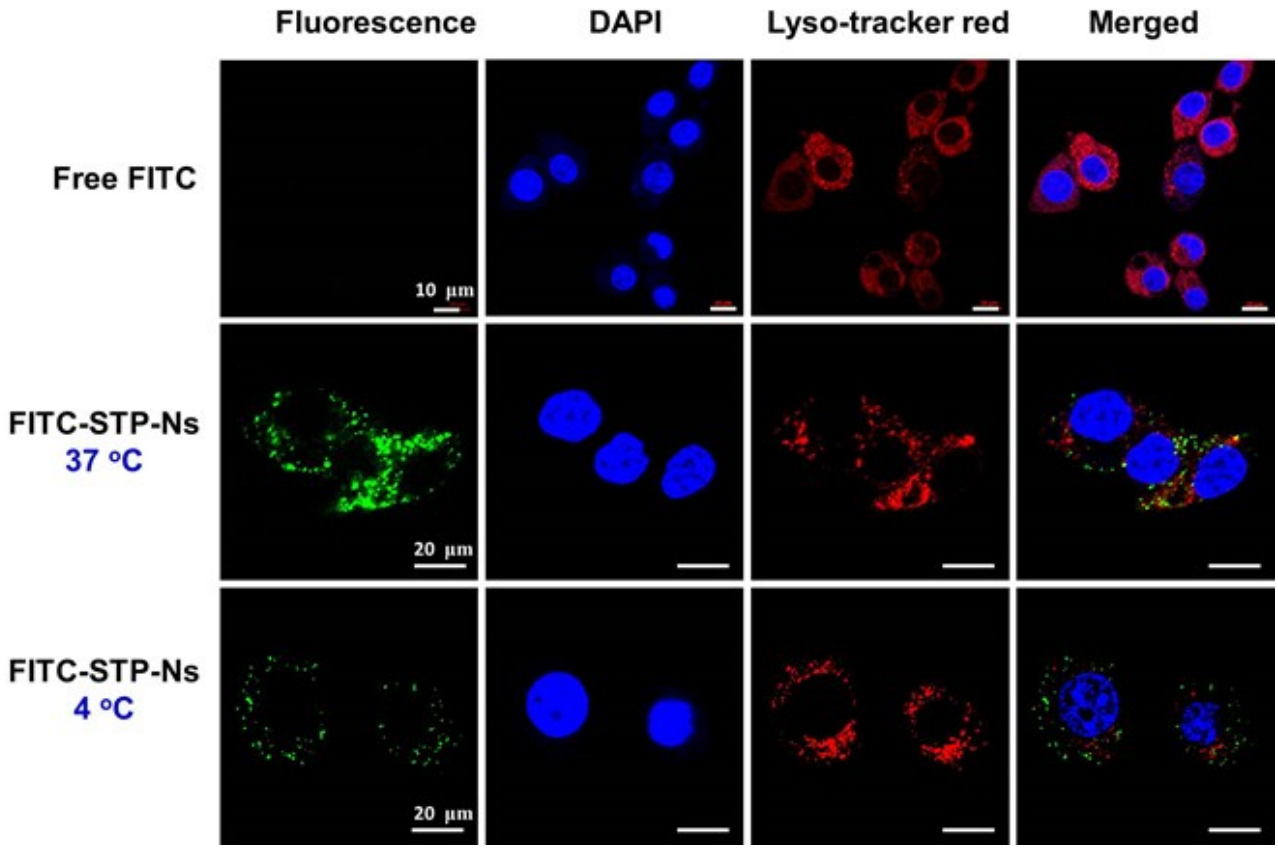

Figure S4. CLSM images of Caco-2 cells cultured with FITC-STP-Ns for 1 h at 37 °C or 4 °C, using free FITC as control. The nuclei (blue area) and lysosomes (red area) were stained with DAPI and Lyso-tracker red, respectively.

Figure S5

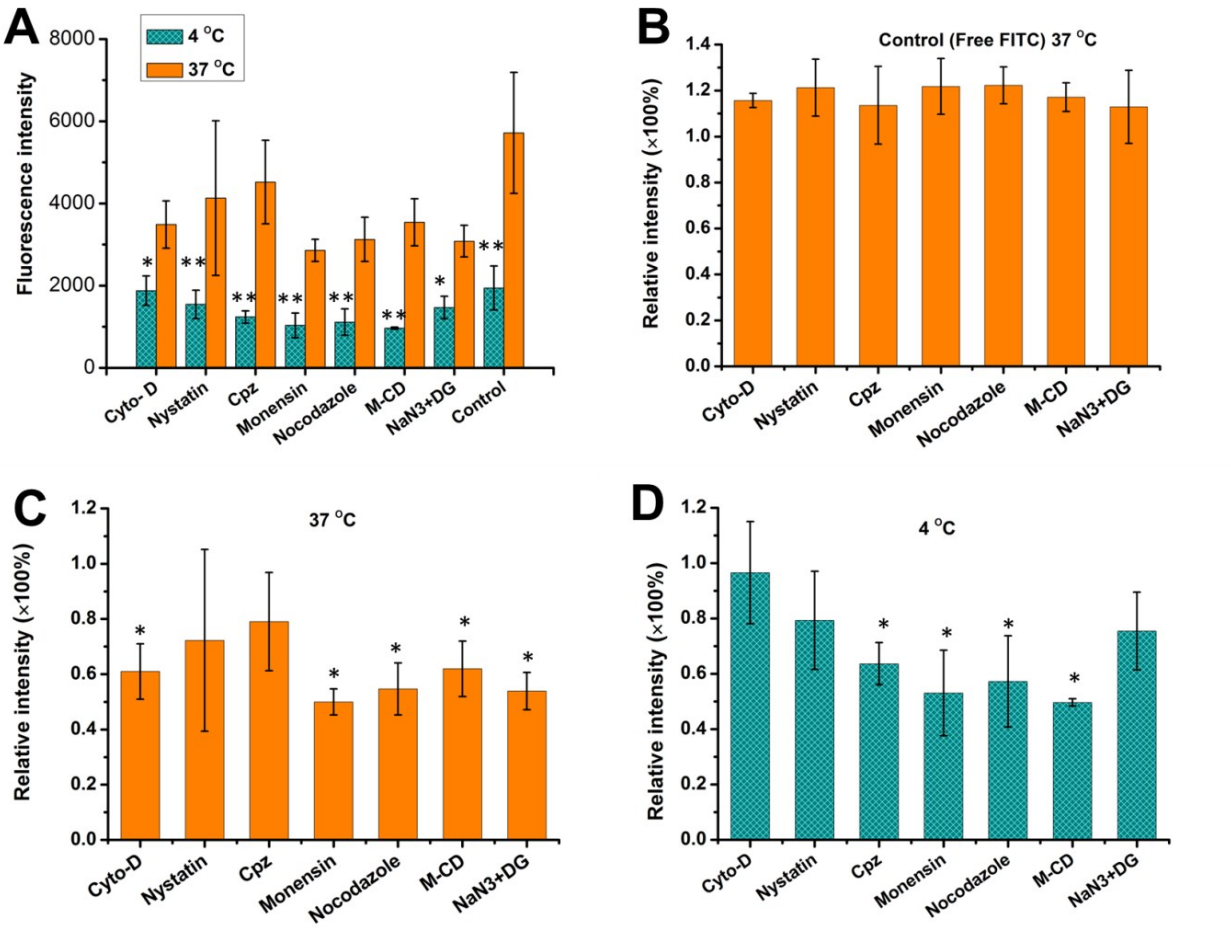

Figure S5. Fluorescence intensity of Caco-2 cells incubated with inhibitors for 30 min before administration of (**A**) FITC-STP-Ns or at 37 °C or 4 °C and relative intensity with respect to control at (**C**) 37 °C or (D) 4 °C (n = 5). (**B**) The control was free FITC. The inhibitors, Cyto-D, nystatin, Cpz, monensin, nocodazole, M-CD, and NaN_3+DG, block macropinocytosis, caveolin internalization, microtubule-related internalization, clathrin-mediated endocytosis, microtubule-related internalization, lysosome-mediated internalization, cholesterol-dependent internalization, and energy-dependent mechanisms of uptake, respectively. *, $p < 0.05$ and **, $p < 0.01$.