Supplementary Information

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

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Table S1

<table>
<thead>
<tr>
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<th>Particle size (nm)</th>
<th>PI</th>
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<tbody>
<tr>
<td>Apical medium</td>
<td>157.60</td>
<td>0.269 ± 0.019</td>
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<tr>
<td>Basolateral medium</td>
<td>158.20</td>
<td>0.338 ± 0.023</td>
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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 °C for 2 h.
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. 2D structure of protein-drug complex
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. 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. 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 NaN₃+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.