Electronic Supplementary Material (ESI) for Lab on a Chip
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and vacuum-dried at room temperature. The prepared Hydrophobically modified chitosan (HMCS) was analyzed by $^1$H NMR (Bruker 400 MHz).

The degree of substitution (DS) of palmitoyl groups on chitosan was determined using the ninhydrin assay.$^2$ HMCS was dissolved in an aqueous acetic acid and then 0.5 mL of 4M acetic acid/acetate buffer (pH= 5.5) was added into 0.5 mL of the prepared solution. 1 ml of ninhydrin reagent (Sigma–Aldrich) was then added and test tubes were placed in a boiling water bath for 20 min. The solutions were cooled and their absorbance at 570 nm was read. The unmodified chitosan solution was used as a control and the acetic acid/acetate buffer was used as a blank.$^1$

Microfluidic devices were fabricated with poly(dimethylsiloxane) (PDMS) using a standard micromolding process. To make the master molds, silicon wafers were spincoated with SU-8 50 photocurable epoxy to a thickness of 60 μm. Baking, lithography, and development procedures were performed at the EPFL center for micronanotechnology (CMi) to obtain negative microchannels on the wafer. The wafers were annealed at 150 °C to eliminate surface cracks in the SU-8. The resulting mold, after thermal annealing, was coated with a self-assembled monolayer of trimethylethoxy silane by vapor exposure for 40 min. The SAM prevents sticking of the PDMS to the mold. PDMS (Sylgrad 184) monomer and curing agent were mixed in a weight ratio of 10:1, pored over the mold, degassed in desiccators and cured in an oven at 80 °C for 1 h. After curing, the PDMS was removed from the mold and in-/outlet holes were punched using a 150 μm diameter punch. The PDMS component was then bonded to a glass slide using oxygen plasma (100 mW, 1 min). The PDMS based microfluidic device had two inlets for water with a pH of 9, one for an aqueous solution of HMCS with a pH of 5.5, and one outlet. The water
stream was split into two in order to achieve two water streams at the flow focusing (T-) junction. The mixing channel was 150 μm wide, 60 μm high and 1 cm long.

Moreover, the mixing time, $\tau_{\text{mix}}$, can be estimated from the diffusion timescale\(^3\), as is shown in Equation S1,

$$\tau_{\text{mix}} \approx \frac{w_f^2}{4D} \approx \frac{w^2}{9D} \frac{1}{(1+1/R)^2} \quad (S1)$$

where, $w$ is channel width (150 μm), $D$ is $10^{-9}$ m\(^2\).s\(^{-1}\) and $R$ is the ratio of flow rate of the polymeric stream to the total flow rate of basic water ($R=0.03$-0.2). $w_f$ can be estimated as the width of the focused chitosan stream (when $R<1$). The above equation predicts a mixing time in the range of 2.5-75 ms in our case.

For bulk synthesis of HMCS based nanoparticles, polymeric solutions were prepared by dissolving 2.5 mg.ml\(^{-1}\) HMCS in 1% w/v acetic acid solution under stirring condition. The nanoprecipitation of HMCS molecules and formation of nanoparticles were performed by drop-wise addition of 1M NaOH to adjust the pH to 7.4.

In the case of drug loaded nanoparticles, Paclitaxel (Sigma-Aldrich) was dissolved in acidic water (pH 5) and mixed with the polymeric solution. The preparation of nanoparticles is the same as detailed above.

Transmission electron microscopy (TEM; CM200-FEG-Philips) was used to characterize the HMCS based nanoparticles. A dilute suspension of nanoparticle was prepared from which the particles were deposited onto the Cu grid with a carbon film. The particle shape and sizes were
characterized via diffraction (amplitude) contrast and, for crystalline materials, by high resolution (phase contrast) imaging. The TEM used a LaB6 source operating at 100 kV accelerating voltage. The images were characterized using ImageJ software with at least 20 different measurements.

Dynamic light scattering (DLS) as well as zeta potential measurements were performed using a Zetasizer (Zetasizer 3000HS, Malvern Instruments Ltd., Worcestershire, UK) in backscattering mode at 173° for water diluted systems.

Table S1. Polydispersity index (PDI) for HMCS nanoparticles with and without paclitaxel drug.

<table>
<thead>
<tr>
<th>Flow ratio</th>
<th>Time of mixing (ms)</th>
<th>DS I No drug</th>
<th>DS I With drug</th>
<th>DS II No drug</th>
<th>DS II With drug</th>
<th>DS III No drug</th>
<th>DS III With drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.031</td>
<td>2.5</td>
<td>0.120</td>
<td>0.156</td>
<td>0.112</td>
<td>0.138</td>
<td>0.108</td>
<td>0.145</td>
</tr>
<tr>
<td>0.050</td>
<td>6.2</td>
<td>0.122</td>
<td>0.168</td>
<td>0.121</td>
<td>0.190</td>
<td>0.091</td>
<td>0.158</td>
</tr>
<tr>
<td>0.0758</td>
<td>13.3</td>
<td>0.183</td>
<td>0.189</td>
<td>0.140</td>
<td>0.187</td>
<td>0.122</td>
<td>0.185</td>
</tr>
<tr>
<td>0.100</td>
<td>22.6</td>
<td>0.192</td>
<td>0.192</td>
<td>0.153</td>
<td>0.172</td>
<td>0.122</td>
<td>0.198</td>
</tr>
<tr>
<td>0.200</td>
<td>76.1</td>
<td>0.161</td>
<td>0.208</td>
<td>0.154</td>
<td>0.182</td>
<td>0.113</td>
<td>0.198</td>
</tr>
<tr>
<td>Bulk mixing</td>
<td>NA</td>
<td>0.720</td>
<td>0.936</td>
<td>0.613</td>
<td>0.605</td>
<td>0.560</td>
<td>0.805</td>
</tr>
</tbody>
</table>

Turbidity measurement:

The transmittance of the prepared samples was measured at room temperature (20 °C) with a Shimadzu UV mini 1240 UV/visible spectrophotometer with a wavelength of 550 nm. The turbidity ($\tau$) was calculated from the transmittance using Lambert-Beer’s law$^{4-5}$:

$$\tau = -\frac{1}{L} \ln \left( \frac{I_t}{I_0} \right) \quad (S2)$$
where $L$ is the light path length in the sample cell (1.0 cm quartz cuvette), $I$, is the intensity of the light transmitted through the sample, and $I_0$ is the intensity of the light transmitted through the solvent (deionized water). Measurements were performed at least triplicate, and the mean values are reported in Table S2.

Table S2. Turbidity values for HMCS nanoparticles in comparison with bulk synthesized nanoparticle.

<table>
<thead>
<tr>
<th>Flow ratio</th>
<th>Time of mixing (ms)</th>
<th>DSI (cm$^{-1}$)</th>
<th>DSII (cm$^{-1}$)</th>
<th>DSIII (cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.031</td>
<td>2.5</td>
<td>0.146</td>
<td>0.21</td>
<td>0.262</td>
</tr>
<tr>
<td>0.050</td>
<td>6.2</td>
<td>0.132</td>
<td>0.187</td>
<td>0.258</td>
</tr>
<tr>
<td>0.0758</td>
<td>13.3</td>
<td>0.098</td>
<td>0.151</td>
<td>0.223</td>
</tr>
<tr>
<td>0.100</td>
<td>22.6</td>
<td>0.085</td>
<td>0.127</td>
<td>0.174</td>
</tr>
<tr>
<td>0.200</td>
<td>76.1</td>
<td>0.058</td>
<td>0.096</td>
<td>0.152</td>
</tr>
<tr>
<td>Bulk mixing</td>
<td>NA</td>
<td>0.047</td>
<td>0.068</td>
<td>0.094</td>
</tr>
</tbody>
</table>

To determine the in vitro drug release profile, lyophilized PTX-loaded nanoparticles (1 mg) were dispersed in 1 mL of phosphate buffered saline (1X PBS, pH 7.4). The solutions were placed into a 3 kDa molecular weight cut-off dialysis cartridge (Thermo Scientific, Rockford, IL). The cartridge was immersed in 1 L PBS and gently shaken in a 37°C water bath. At predetermined intervals, buffered solutions were collected and replaced with an equivalent volume of fresh PBS.

The PTX concentration was measured with high-performance liquid chromatography (HPLC) by mixing the 1 ml of sample with 1 ml of acetonitrile as follows: A reverse phase C18 column was used as the stationary phase. The mobile phase consisted of acetonitrile:water (60:40 vol/vol). Separation was carried out at a flow rate of 1 mL.min$^{-1}$. PTX was detected at a wavelength of
230 nm. This method was evaluated over a linear range of 1–100 \( \mu g.ml^{-1} \). In this range, the percent deviation from theoretical value was found to be less than 5% and the R-square values remain less than 4% using clean PTX standards.

The PTX concentration in the solution was corrected for sampling effects according to following equation:

\[
C_n' = C_n[V_T/(V_T-V_S)](C_{n-1} / C_{n-1})
\]  

where \( C_n' \) is the corrected concentration of the \( n^{th} \) sample, \( C_n \) is the measured concentration of PTX in \( n^{th} \) sample, \( C_{n-1} \) the measured concentration of the \((n-1)^{th}\) sample, \( V_T \) the volume of receiver fluid and \( V_S \) the volume of sample drawn (1 ml).

Encapsulation efficiency of nanoparticles and loading efficiency were determined by applying the following equations:

PTX Loading content = (Weight of loaded PTX / Weight of nanoparticle)\( \times 100 \)  

PTX Loading efficiency = (The amount of PTX in the nanoparticles/ total amount of nanoparticle weight) \( \times 100 \)
Fig. S1. Effect of flow ratio on paclitaxel (PTX) loading content for three different substitution degrees of palmitoyl groups (Mean± SD, n =5 independent experiments).

Supplementary References: