Supplementary Information

Chemical and Mechanical Modulation of Polymeric Micelle Assembly

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Figure S1. ¹H-NMR spectrum for polysuccinimide (PSI). The peak areas at 5.25 ppm, 3.2 ppm, and 2.71 ppm are approximately equal, confirming the successful synthesis of a polymer with succinimide rings. The tan area corresponds to d6-DMSO.



Figure S2. ¹H-NMR spectrum for oligovaline-PHEA-C₁₈ (DS_{C18} = 20 %). Peaks are denoted for the PHEA backbone (4.5 ppm), the octadecyl chain (0.85 ppm), and valine (1.02 and 1.12 ppm). Note that the tan shaded area denotes the presence of water. The red curves represent the approximate areas integrated to quantify the degree of substitution of the octadecyl chain, or the number ratio of valine groups to PHEA units. Integrated area at 4.55 ppm: 0.034; integrated area at 1.03 ppm: 0.0023; integrated area at 0.85 ppm: 0.023.



Figure S3. Structure of valine n-carboxyanhydride (valine-NCA) used to prepare oligovaline chains.



Figure S4. ¹H-NMR spectrum for valine-NCA. The peaks at approximately 0.95 ppm correspond to the protons on the methyl groups on the isopropyl group.



Figure S5. Intensity ratio ($I_{337 nm}/I_{334 nm}$) versus oligovaline-PHEA-C₁₈ concentration. A polymer solution incubated with pyrene was excited from 300 to 360 nm, and the emission was collected at 395 nm. The polymer concentration at the inflection point (i.e., the intersection of the horizontal line and the upward sloping line) is defined as the critical micelle concentration (CMC).



Figure S6. Schematic of polymer with 11 units used to approximate PHEA molecules for molecular simulation.



Figure S7. Effects of oligovaline on the solubility of PHEA in DMF at higher DS_{C18} values. Images of PHEA polymers dissolved in DMF at 30 mg/mL: (i) NH₂-PHEA-C₁₈ (DS_{C18} =40%); (ii) Oligovaline-PHEA-C₁₈ (DS_{C18} =40%).

	DS _{C18} (%)	N _{PHEA/Val}	Solubility in DMF at 30 mg/mL
NH ₂ -PHEA-C ₁₈	20	0	Insoluble
NH ₂ -PHEA-C ₁₈	40	0	Insoluble
Oligovaline-PHEA-C ₁₈	20	0.01	Soluble
Oligovaline-PHEA-C ₁₈	40	0.01	Soluble
Oligovaline-PHEA	0	0.04	Soluble

Table S1. The solubility of different PHEA polymers formed with varying amounts of octadecyl chains and oligovaline chains.



Figure S8. Transmission electron microscopy (TEM) images of polyaspartamide nanoparticles created as additional controls. Particles were fabricated via off-chip precipitation using oligovaline-PHEA (a) or NH₂-PHEA-C₁₈ (DS_{C18} = 20 %) (b). Scale bar denotes 100 nm.



Figure S9. Transmission electron microscopy (TEM) images of oligovaline-PHEA- C_{18} (DS_{C18}=20%) micelles formed with off-chip mixing (a), with the microfluidic mixer at FRR-10 (b), and with the microfluidic mixer at FRR-20 (c). The white scale bars represent 200 nm.



Figure S10. Scanning electron microscopy (SEM) images of micelles formed with microfluidic mixer at FRR-10 (a) and FRR-20 (b). Both white scale bars correspond to 100 nm.



Figure S11. Atomic force microscopy (AFM) images of micelles prepared with microfluidic mixer at FRR-10 (a) and FRR-20 (b).



Figure S12. The hydrodynamic diameter of micelles prepared with microfluidic mixer. The diameter was measured over the course of 1 week using dynamic light scattering (DLS). The micelles were incubated in PBS. Values and error bars represent the mean and standard deviation from at least 3 measurements per sample, respectively.

Supplementary Information Protocols

Synthesis of Polysuccinimide (PSI) First, 50 g of L-aspartic acid was dissolved in 160 g of warm sulfolane under vigorous stirring. The temperature was gradually brought to 170 °C under nitrogen. Then, 1.1 mL of 85% phosphoric acid (Fisher) was added to the reaction mixture as a catalyst. A glass outlet was added to the reaction flask to remove excess water from the reaction. After reacting for about 7 hours, the reaction mixture was cooled to room temperature. The reaction mixture was then precipitated with 200 mL of methanol (EMD), and then several times with 200 mL of DI water. The excess water from each washing step was tested with a pH strip (Hydrion). Here, a neutral pH reading confirmed removal of any impurities. Afterwards, the precipitate was loaded into a dialysis bag, and then dialyzed against DI water (MWCO 12,000-14,000, Fisherbrand). The dialysis water was changed approximately every 12 hours. Afterwards, the precipitate was removed from the dialysis bag, frozen at -20 °C, and then lyophilized to form a dry powder (Labconco Freezone 6).

Synthesis of Valine-N-carboxyanhydride (valine-NCA) First, 1.2 g of L-valine was dissolved in 12 mL of tetrahydrofuran (THF). Separately, 1.2 g of triphosgene was added to 2 mL of THF, and then added to the L-valine solution dropwise. The reaction mixture was then kept at 40 °C for 3 hours under nitrogen. Afterwards, the reaction mixture was cooled and then vacuum-filtered to remove any insoluble material. The filtrate was then added to 300 mL of hexane, and then crystallized at -20 °C.

Synthesis of Oligovaline-PHEA-C₁₈ and PHEA-C₁₈-NH₂ Labeled with Fluorescein Isothiocyanate (FITC). Briefly, oligovaline-PHEA-C₁₈ and PHEA-C₁₈-NH₂ were dissolved in DMF. Then, a solution of fluorescein isothiocyanate (FITC) was added dropwise to the polymer solution. The mass ratio of PHEA polymer to FITC was kept at approximately 1:0.0006. The reaction continued for 24 h at room temperature (for oligovaline-PHEA-C₁₈) or 24 h at 60 °C (for PHEA-C₁₈-NH₂). Afterward, the reaction mixtures were dialyzed (MWCO 3,500, Fisherbrand) against DI water for 48 hours; fresh DI water was added at least twice.

Mold Fabrication for Microfluidic Devices Molds for replicating polydimethylsiloxane (PDMS) microfluidic devices were fabricated using standard photolithography procedures. Three-inch silicon wafers (University Wafer) were cleaned by rinsing with acetone then isopropanol and dried by blowing nitrogen over the wafers. Cleaned silicon wafers were then heated on a hot plate at 115 °C for 3 min and cooled by blowing nitrogen over the wafers. SU-8 2050 photoresist (MicroChem Corp.) was spin-coated onto the wafers to a final thickness of roughly 100 μ m. The SU-8 coated silicon wafers were baked on hot plates for 5 min at 65 °C, then 17 min at 95 °C, and finally 2 min at 65 °C. Wafers were then placed inside a UV exposure system (OAI). A transparency mask, made using Adobe Illustrator CS5 (Adobe Systems Incorporated,) and printed by Fineline Imaging (Fineline Imaging), was placed on top of the SU-8. A PL-360LP filter (Omega Optical, Inc.) was placed on top of the transparency to reduce air gaps between the mask and SU-8, and reduce T-topping from short wavelength (<350 nm) light. The wafers were placed on a hot V light (5.44 mW/cm²) for 47 seconds. The UV exposed SU-8 wafers were placed on a hot

plate and the temperature was ramped from room temperature to 55 °C at 2 °C/min. Wafers were then baked for 2 h at 55 °C, allowed to cool to room temperature, and then developed by gently swirling in propylene glycol monomethyl ether acetate (PGMEA) for about 10 min. Developed wafers were rinsed with PGMEA, then isopropanol, and dried by blowing nitrogen over the wafers. Wafers were then coated with perfluorodecyl-1H,1H,2H,2H-trichlorosilane (Gelest) via vapor deposition to prevent adhesion of PDMS to the mold.

Nanoparticle-Extruding Microfluidic Device Fabrication PDMS replicates were made by mixing RTV 615 (Momentive Performance Materials,) or Sylgard 184 (Dow Corning) base and curing agent at a 10:1 ratio. The mixture was degassed in a vacuum desiccator for ~20 minutes, poured on a mold inside a petri dish, and placed in an oven at 65 °C for 2 hours to cure the PDMS. The PDMS was peeled off the mold and holes were punched at the inlets and outlet using 19 gauge hypodermic tubing with a beveled end. A glass slide (Thermo Fisher Scientific Inc.) was cleaned with the Alconox solution, rinsed with water (18.0 M Ω cm), and dried with nitrogen. Oxygen plasma was used to activate the PDMS and glass slide surfaces to ensure stable bonds between PDMS and glass. Then, the PDMS replicate was pressed against the glass slide and incubated in an oven at 65 °C overnight.

AFM Imaging of PHEA Nanoparticles Atomic force microscopy (AFM) measurements were carried by imaging particles suspended in the HPLC-grade water. First, the particle suspension was dropped on the freshly cleaved mica sheet. The particles were imaged with the intermittent contact mode, using a Nanowizard® II (JPK Instruments Ltd) fitted with SiNi cantilevers (BudgetSensors®). This study used cantilevers with a spring constant of 0.27 N m⁻¹.

SEM Imaging of PHEA Nanoparticles PHEA nanoparticles were suspended in water at 0.75 - 1.5 mg/mL. Then, 20 uL of the nanoparticles suspension was dropped onto 200-mesh copper grids placed on top of a filter paper. Excess media was wicked away by a separate filter paper. Then, the residual water was removed by exposing samples to air overnight before imaging. Images were captured with FE-SEM (Hitachi S4800) at the accelerating voltage of 5.0 kV and the emission current of 10 μ A.

COMSOL Simulation to Characterize On-Chip Mixing

"Single-phase laminar flow" and "transport of diluted species" modules were coupled in COMSOL in order to solve the Naviér-Stokes (N-S) equation and convective-diffusion equation, respectively for incompressible fluid. The governing equations are as follows:

Naviér-Stokes equation:

$$\rho(\nu \cdot \nabla)\nu - \nabla \cdot \eta(\nabla \nu + (\nabla \nu)^T) + \nabla p = 0$$
(S1)

$$\nabla \cdot \nu = 0 \tag{S2}$$

Convective-Diffusion equation:

$$\mathcal{D}\nabla^2 c - \nu \cdot \nabla c = 0 \tag{S3}$$

In the above equations, ρ denotes density (kg/m³), v is the velocity vector (m/s), η denotes viscosity (Pa-s), p equals pressure (Pa), D denotes the diffusion coefficient (m²/s) and c represents the concentration (mol/m³). The following fluid properties were used in the simulation; water – density: 1,000 kg/m³, dynamic viscosity: 0.89 mPa-s, concentration: 55,400 mol/m³; DMF – density 944.6 kg/m³, dynamic viscosity: 0.864 mPa-s, concentration: 12,900 mol/m³. The diffusion coefficient was kept as $1x10^{-9}$ m²/s for both water and DMF, which is in agreement with the common values found in literature.¹

Simulations were performed for three different flow rate ratios (FRRs), 5, 10, and 20, while keeping the total volumetric flow rate constant (140 μ L/min). A triangular mesh with 60,260 elements and calibrated for fluid dynamics was used for all the simulations.

The Reynolds number was calculated using the following formula:

$$Re = \frac{vl}{v}$$
(S4)

where v, l, and v are total linear flow velocity, characteristic length scale, and kinematic viscosity in the outlet channel, respectively. Reynolds number for the off-chip mixing was calculated using the equation for a stirred tank.²

$$Re = \frac{\rho N D^2}{\mu}$$
(S5)

Whereby ρ is the density of DMF (0.9446 g/mL), μ is the viscosity of DMF (0.846 mPa-s), N is the rotational speed (1,000 rpm), and D is the diameter of the agitator (12.7 mm).

References for Supporting Information

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- (2) Schmidt, F. R. Optimization and Scale up of Industrial Fermentation Processes. *Appl. Microbiol. Biotechnol.* **2005**, 68 (4), 425–435.