**Supplementary Methods**

**Microfluidic Device Mold Fabrication and Characterization**

Molds for the SHM were printed using a Miicraft Ultra 50 3D Printer, using MasterMold Resin for PDMS (ResinWorks3D). For characterization experiments, molds were characterized using a Keyence VHX-5000 microscope using a VH-Z20R lens to measure the SHM and herringbone dimensions. The full characterization data are included in Supplementary Tables S1-S4.

For device fabrication, printed molds were washed in a bath of isopropyl alcohol for 10 minutes, cured under UV light for another 10 minutes and then baked for 4 hours at 130 °C. Molds were then treated via the vapor deposition of N-decyltrichlorosilane for 30 minutes to prevent attachment of PDMS to the master mold [1]. Microfluidic devices were then fabricated with standard soft lithography techniques [2]. Polydimethylsiloxane (PDMS), obtained as commercially available Sylgard 184 (Dow Chemical), was mixed at a 10:1 ratio of PDMS to cross-linker, poured in the treated molds, degassed for approximately 2 hours, then cured at 60-80 °C for 4h, and removed from the mold. To prepare the microfluidic chambers, the PDMS layer was bonded to a standard glass slide. Both the PDMS and a glass slide were treated in an ozone cleaner for 10 minutes, and the PDMS was then manually pressure sealed onto the glass slide and post-cured at 60 °C for at least 15 minutes.

**Mixing Tests**

The quality of mixing was assessed by observing via quantitative fluorescence microscopy the steady-state flow and mixing of two input streams, one containing a fluorescent marker and the other containing neat water. In detail, a fluorescent solution of 70 kDa fluorescein isothiocyanate (FITC)-dextran at a concentration of 0.30 µM suspended in water was injected into Input 1, while water was injected into Input 2 (Figure 1). A 2-channel syringe pump (NE-4002X, New Era) was used to ensure equal flow rates, which were varied from 0.5 µL min-1 to 20 µL min-1 (Reynold’s Number *Re*: 0.01-0.4, Peclet Number *Pe*: 200-8000). The solutions within the channels of the microfluidic devices were imaged with an Olympus IX53 inverted microscope using a UPlanFL N lens with a 10x objective, and images were recorded using a Flir CMLN-13S2M-CS camera, at a magnification of 0.367 µm per pixel. Videos of 10 frames each were recorded at 15 frames per second after the interface between the two streams had stabilized, indicating that steady-state flow had been reached. This typically occurred after ~5-6 control volumes of 0.01 mL in each stream had passed through the device, with waiting times between 2 and 30 minutes.

We note that the presence of the 90° bends in the design can also promote mixing. In order to assess the relative importance of the bends and SHM elements in our work, we calculate the mixing quality factor, α, proposed by [3] and defined as:

$$α=1-\sqrt{\frac{σ^{2}^{ }}{σ\_{max}^{2}}}$$

where $σ^{2}^{ }$ is the variance of the concentration within a cross section of the channel, and $σ\_{max}^{2}$ is variance within a cross section of the channel before any mixing has occurred (i.e., at the interface where the two streams are introduced). Thus *α* varies from 0 to 1, with a value of 1 representing perfect mixing. For *Re* = 0.4 and a channel cross sectional area of 500 µm x 500 µm, the *α* obtained after a single 90° bend in the channel was approximately 0.13 [3]. By contrast, we calculated *α* after a single cycle of mixing at *Re* = 0.4 and for a similar channel dimension to be 0.8, an approximately 6-fold increase in mixing quality due to the SHM. Thus, we expect that the presence of the 90° bends in this SHM design improves mixing, but that they likely do not dominate the mixing response.

**Mixing Analysis**

The *Re* and *Pe* values were calculated as follows:

$Re= \frac{uH}{v}$,

 $Pe= \frac{uL}{D}$,

where $u$ is the average velocity defined by $u=q/WH$ and $q$ is the volumetric flow rate, $ H$ is the channel height, ** is the kinematic viscosity of water, $L$ is a characteristic length, given here by half of the channel width: $W/2$, and *D* is the Stokes-Einstein diffusivity of the FITC dextran molecules. *D* is calculated by the following formula:

$D= \frac{k\_{B}T}{6πηr}$,

where *kB* is the Boltzmann constant, *T* is solution temperature, $η$ is solution shear viscosity, and *r* is the molecular radius (obtained from Sigma Aldrich).

All image processing was performed using MATLAB R2019a. Videos were uploaded into MATLAB and the edges of the channel were detected. Edge detection was performed by calculating the gradient in the *x* direction at each point (channel edges oriented such that they were roughly running vertical, in the *y* direction), and edge points were identified as those with the largest gradient in each row of the image. To avoid possible irregularities in intensity values near the edge of the channel, the intensity data within 50 pixels of the channel edge on either side were ignored. The resulting truncated 2D intensity data were used to generate surface plot profiles, which allow for a qualitative analysis of the fluorescence intensity as a function of position within the channel. To enable more quantitative comparison, 1D intensity profiles were generated by averaging the intensity data from 50 rows of pixels at each position across the width of the channel and these averaged values smoothed. The fluorescence data within a single image frame were normalized by dividing each point by the maximum intensity value, yielding values ranging from 0 to 1, enabling comparison between mixing tests (Fig. S4). The fluctuations in normalized intensity were evaluated using the coefficient of variation (*CV*), which is defined in this context as the standard deviation of the normalized fluorescence intensity divided by the average of the normalized intensity values of the 1D intensity profiles. The *CV* values were then used as a metric to determine the quality of mixing. To confirm that the flow had reached steady-state, and that CV values did not vary over the course of the video, we calculated the CV value from the first and last frames of each video, and minimal variation was observed. Any profile with a CV < 0.1 was classified as well mixed [4].

The error in the observed CV values was estimated as follows:

$$\left(\frac{δ\overbar{CV}}{\overbar{CV}}\right)^{2}=σ\_{diff}^{2}\left(\frac{1}{σ\_{1}^{2}}+\frac{1}{σ\_{2}^{2}}+\frac{1}{\overbar{I\_{1}^{2}}}+\frac{1}{\overbar{I\_{2}^{2}}}\right)$$

$δ\overbar{CV}$ is the error in the reported CV values$, \overbar{CV}$, which are averages of the CV values calculated from intensity distribution of the first and last frames of each video. $σ\_{diff}$ refers to the standard deviation of the difference image obtained by subtracting the intensity values of the last frame from the first, and $\overbar{I\_{i}}$ and $σ\_{i}$ refer to the mean and standard deviation in intensity values of a single frame.

**References (Supplementary Material)**

1. Razavi Bazaz S, Kashaninejad N, Azadi S *et al.* Rapid Softlithography Using 3D-Printed Molds. *Advanced Materials Technologies*, 4(10), 1900425 (2019).

2. Qin D, Xia Y, Whitesides GM. Soft lithography for micro- and nanoscale patterning. *Nature Protocols*, 5(3), 491-502 (2010).

3. Kockmann N, Engler M, Haller D, Woias P. Fluid Dynamics and Transfer Processes in Bended Microchannels. *Heat Transfer Engineering*, 26(3), 71-78 (2005).

4. Williams MS, Longmuir KJ, Yager P. A practical guide to the staggered herringbone mixer. *Lab on a Chip*, 8(7), 1121-1129 (2008).