# Supporting information

#### A. Pressure drop measurements

Figure S1 exhibits the pressure drop measurements performed with a pressure driven pump. A highly viscous wormlike micellar solution (zero-shear viscosity  $\eta_0$  of about  $2 \times 10^{-3}$  Pa.s) composed of 100 mM cetyltrimethyl ammonium bromide CTAB and 60 mM of sodium salicylate NaSal is used as the test fluid. A minimum of 700 mBar was necessary to start the flow of such viscous solution. The pressure drop was increased in steps of 100 mBar, taking into account the time needed to measure a stable pressure. The dimensions of the channel, and thus the deformation, were not measured. We determine the performance of the channel based on the presence of leakage, the expansion of the PI covers and/or the appearance of defects on the PI/NOA or PI/glass interface. The burst of the channel is very prominent in the case of highly viscoelastic solutions, as even small channel defects lead to fluid expansion and leakage.

## B. Detailed microfabrication process

#### Hard mold and flexible stamp

The description of the microfabrication process is schematically represented in the Fig. 1 of the main text. A silicon hard mold (Fig. 1a) was fabricated by standard photolithography of SU-8 negative resist (Nano SU-8 100, MicroChem Corp., MA, USA). For microchannels thinner than 200  $\mu m$ , one layer of resist was spincoated onto a silicon wafer. The spinning speed and soft-baking temperatures were employed as indicated by the supplier. For microchannels thicker than 200  $\mu m$ , a double layer of SU-8 was spin-coated. In this case, the soft-baking temperature was reached with slow ramps of 2°C/min, as we observed that quick temperature variations cause the resist layer to shrink unevenly, leading to rough surfaces. After soft-baking, the coated silicon wafer was exposed to UV light (MA6, Suess MicroTech, Garching, Germany) through the dark field photomask (JD Photo-Tools, Oldham, UK). After the post-exposure baking steps, the development of the hard mold with mrDev600 (Micro Resist Technology GmbH, Berlin, Germany) was performed in ultrasound bath for 10 min. The final hard mold with negative patterns was rinsed with 2-propanol. The soft lithography process (in Fig. 1b-d) produces a flexible stamp with positive patterns made out of poly(dimethilsiloxane) (PDMS, Sylgard 184, Dow Corning Corp., Midland, USA). For that, PDMS and crosslinker were mixed at a ratio of 10:1 to create firm, but flexible stamps. The mixture was poured onto the hard mold (Fig. 1b) and backed for 17 minutes at 80°C. Afterward, the patterned PDMS replica was removed from the hard mold (Fig. 1c) and cut into the flexible

stamp shape represented in Fig. 1d. Notice the hard mold and the flexible stamps can be reused to fabricate numerous chips, following the next steps.

# PI/NOA/PI chips

Polyimide (PI) films with thickness of 25  $\mu$ m (Goodfellow Corp., Cambridge, UK) were cut into  $5 \times 4$  cm<sup>2</sup> and cleaned with ethanol. A flat metallic surface was used as a base for the PI film, onto which about 0.2 ml of Norland Optical Adhesive (NOA 81, Norland, Cranberry, USA) was poured as shown on Fig. 1e. The flexible stamp is used to spread the NOA uniformly, helping also to remove bubbles and other defects. The whole metallic base was exposed to a UV light source of  $25.5 \text{ mW/cm}^2$ with a distance of 7.5 cm (Model 30, OAI Corp., San Jose, USA). The NOA layer was fully cured through the PDMS stamp during 30 seconds (Fig. 1g). Figure 5h represents the open microchannel structure, which has the PI film as base and the stamped NOA layer forms the side walls as a spacer. Additionally, the NOA layer was treated by corona plasma discharge (BD-20AC, Electro-Technic Products Inc., Chicago, USA) to promote the bonding between NOA and PI, as schematically represented in Fig. 1h. Corona plasma discharge causes the surface to oxidize, increasing the bonding strength between different materials<sup>1</sup>. Holes for the inlet and outlet tubing were punched (Fig. 1i) with a blunt syringe needle. A second PI film was also oxidized by corona plasma and used to cover the open channel of the chip. Notice that the corona plasma oxidation is fundamental, since a curing step to bond the NOA spacer to the second PI film is not possible, because PI films do not transmit UV light. After the surface oxidation steps, the chips referred to as PI/NOA/PI, could be assembled (Fig. 1). For stronger bonding, we recommend to leave the chips overnight in an oven under low temperature of about  $50^{\circ}$ C with 1-2 kg weight on top before using.

# PI/NOA/glass chips

To fabricate PI/NOA/glass chips, the coronal discharge step (Fig. 1h) was not necessary. Instead, the NOA layer was only partially cured through the PDMS stamp during 7 seconds of exposure to a UV light, as shown in Fig. 1g. This short exposure time was not enough to completely cure the NOA layer, and the PDMS stamp was easily removed. After the holes were punched, a thin microscope cover slip (No. 1, 0.13-0.16 mm thick,  $710\pm10$  mm long, Menzel Glaeser, Braunschweig, Germany) was used to cover the open channel structure of the chip. The whole chip was further exposed to UV light for 120 s with the cover slip facing upwards to permit the complete cure of the NOA layer and its bonding with glass.

# Microfluidic system

A sample holder, as shown in Fig. 11, is necessary to give mechanical resistance to the chips and to connect the inlet and outlet tubing. A sandwich-like holder made out of aluminum and polyether ether ketone (PEEK) was used to connect the chip to the syringe pump through a polytetrafluoroethylene (PTFE) tubing  $(0.30 \times 0.76 \text{ mm})$ , Elveflow, Paris, France). O-rings are placed around the tubing and the punched holes of the chip to avoid leakage. All scanning-SAXS measurements of microfluidic flows were performed using a pulsation-free syringe pump (Nemesys, Cetoni, Korbussen, Germany) and 1 ml glass syringes (Gastight, Hamilton, Reno, USA). A micropump with an integrated high accuracy piezo-pressure controller up to 1900 mbar (AF1, Elveflow, Paris, France) was used to determine the maximum pressure reached inside the chips.

## C. SAXS measurements in capillaries

Static measurements were performed in boron silicate glass capillaries (Hilgenberg, Malsfeld, Germany) with outer diameter of 1 mm and wall thickness of 0.01 mm. The X-ray beam was focused to  $200 \times 200 \ \mu m^2$  and the photon energy set on 12.4 keV ( $\lambda \approx 1$  Å) with sampleto-detector-distance of 2.14 m. Two-dimensional scattering patterns were collected by a Pilatus 2M detector  $(1475 \times 1679 \text{ pixels, pixel size: } 172 \times 172 \,\mu\text{m}^2)$ . The X-ray beam was focused on the detector, and different positions along the long axis of the capillary were measured. Ten scattering patterns were collected consecutively at the same position, each one with exposure times of 0.1 s followed by a pause of 0.1 s. Then the next point, located 5 mm from the initial one, was measured. A total of 10 points (each one composed by 10 short measurements) were scanned 3 times, resulting in a total of 300 scattering patterns, which were monitored for radiation damage and finally averaged to improve the statistics.

# D. Scanning-SAXS data analysis

High spatially resolved SAXS scans of extended areas generate large amount of data, thus the analysis should be automated as much as possible to allow easy interpretation and data reduction. An example of a measured 2D scattering pattern is represented in Fig. S2a. Figure S2b shows a schematic representation of the radial integration over the counts from Fig. S2a, resulting in the curve of scattering intensity I as a function of the scattering vector q. For the data analysis, range of scattering vectors  $\Delta q$  can be selected to intensify the signal generated by specific sample dimensions d, considering that  $q = 2\pi/d$ . For example, if we consider the approximate radius of rodlike particles for the calculation of  $\Delta q$ , the contributions from other dimensions of the particle can be reduced.

For orientation analysis of anisotropic particles under flow, the online analysis method developed by Bunk et al.<sup>2</sup> was employed. However, there are other methods available for the determination of molecular orientation from SAXS data, for example, based on the use of rotation matrices<sup>3</sup>. Figure S2c-d displays a schematic overview of this method, where each 2D scattering pattern is divided into 16 azimuthal segments of  $22.5^{\circ}$  in the chosen  $\Delta q$ . The intensity within these segments is then integrated and normalized based on sample absorption using the relative transmission. The resulting intensity distribution in Fig. S2d is composed by the intensity of 16 azimuthal segments as a function of the azimuthal angle  $\Theta$ .  $I(\Theta)$  is approximated by a cosine function through a discrete Fourier transform, as shown in Fig. S2d. The average scattering intensity of the scattering pattern is represented by the baseline or symmetric amplitude  $a_{sym}$ of the cosine function. In case of anisotropic scattering, the intensity of sample orientation is given by the asymmetric amplitude  $a_{asym}$  of the cosine. As a result, the degree of orientation can be calculated by  $a_{asym}/a_{sym}$ . The phase shift  $\Theta_s$  is directly related to the direction of the scattering pattern orientation, which is always perpendicular to the sample alignment in real space.

## E. Background scattering of the different chips

Figure S4 compares the background signal B of the channel filled with buffer solution to the sample scattering signal S + B from measurements in capillaries at rest (Fig. S4a), and under flow in PI/NOA/PI chips (Fig. S4b) or in PI/NOA/glass chips (Fig. S4c). The sample signals S, after being corrected for background scattering, are compared in the scattering curves in Fig. S4d.

A clear sample signal S is measured from quiescent amyloid fibrils in capillary (see Fig. S4a) after background subtraction. Notice that this signal results from a scattering volume of about  $4 \times 10^{-11}$  m<sup>3</sup>, which is calculated based on the capillary and beamsize dimensions. In the case of microfluidics, the reduction of the beamsize to tens of micrometers yields to the high spatial resolution which is fundamental in the quantification of the influence of confinement and flow on the structure of soft matter. The focused beam leads to scattering volumes of about  $4 \times 10^{-13}$  m<sup>3</sup>, which is  $\approx 100$  times smaller compared to the capillary. The high flux radiation focused to a micro-beam allows for a relatively short acquisition times even with small scattering volumes. This factor is fundamental to investigate dynamic processes in soft matter under flow with high temporal resolution.

In Fig. S4b the scattering of amyloid fibrils, when subjected to flow at 5  $\mu$ l/min in a contraction PI/NOA/PI chip, is measured and averaged along the channel centerline as indicated in the inset in Fig. S4b. The background signal exhibits the characteristic peak of PI at  $q \approx$   $4 \text{ nm}^{-1}$ , which is reduced compared to PI/NOA/glass chip in Fig. S4c, which contains only one layer of PI film. The corrected sample signals in Fig. S4d are normalized by the correspondent exposure time. The  $\Delta q$ corresponds to the mean distance between aligned amyloid fibrils, determined by fitting a rodlike form factor to the scattering curve. Here, it is clear that the sample flowing in PI/NOA/PI chip generates in average stronger signal than in a PI/NOA/glass chip with same thickness, because of the higher background scattering and absorption of glass compared to PI. Additionally, the effect of flow in the scattering curves can be seen for  $q < 0.5 \text{ nm}^{-1}$ . This could be associated with the alignment of amyloid fibrils under flow, since phases with aligned molecules improve the diffraction statistics, leading to additional information about the structural order<sup>4</sup>.

## **References:**

- <sup>1</sup>K. Haubert, T. Drier, and D. J. Beebe, "PDMS bonding by means of a portable, low-cost corona system." Lab on a Chip **6**, 1548– 1549 (2006).
- <sup>2</sup>O. Bunk, M. Bech, T. H. Jensen, R. Feidenhans'l, T. Binderup, A. Menzel, and F. Pfeiffer, "Multimodal x-ray scatter imaging," New Journal of Physics 11, 123016 (2009).
- <sup>3</sup>M. van Gurp, "The use of rotation matrices in the mathematical description of molecular orientations in polymers," Colloid & Polymer Science **273**, 607–625 (1995).
- <sup>4</sup>T. Pfohl, A. Otten, S. Köster, R. Dootz, B. Struth, and H. M. Evans, "Highly packed and oriented DNA mesophases identified using in situ microfluidic X-ray microdiffraction." Biomacromolecules 8, 2167–72 (2007).
- <sup>5</sup>J. Zhao, S. Bolisetty, J. Adamcik, J. Han, M. Fernández-Ronco, and R. Mezzenga, "Freeze-Thaw Cycling induced Isotropic-Nematic Coexistence of Amyloid Fibrils Suspensions," Langmuir **32**, 2492–2499 (2016).
- <sup>6</sup>I. Usov and R. Mezzenga, "FiberApp: An open-source software for tracking and analyzing polymers, filaments, biomacromolecules, and fibrous objects," Macromolecules **48**, 1269–1280 (2015).



FIG. S1. Set and measured pressure drop as function of time. Estimation of the bonding strength based on the pressure drop, which the chips could withstand without deforming, leaking or bursting.



FIG. S2. Scanning-SAXS data analysis. (a) Resulting 2D scattering pattern showing the number of photons recorded per pixel. (b) Schematic representation of the radial integration of the intensity as a function of the scattering vector q, calculated based on the counts recorded by the detector. The selection of a  $\Delta q$  permits the selection of a q-range of interest, which can distinguish between different structures and/or intensify the signal for a specific structural dimension. (c) Schematic representation of the azimuthal integration of the intensity as a function of azimuthal angle  $\Theta$  into 16 segments of 22.5°. Notice that the specific  $\Delta q$  selected from the scattering curve in (b) is considered for the azimuthal integration. (d) Azimuthal plot composed by the 16 segments calculated by azimuthal integration<sup>2</sup>.



FIG. S3. **TEM images of amyloid fibrils having different dimensions prepared at various shearing strengths** and time duration. (a) F1: 0 V. (b) F2: 20 V, 200 rpm, 14 min. (c) F3: 25 V, 1600 rpm, 5 min. (d) F4: 30 V, 3000 rpm, 5 min. (e) F5: 40 V, 4470 rpm, 5 min<sup>5</sup>. (f) Length distribution of F1-5. The solid lines represent the best fit of a log-normal distribution function. The arithmetic average length  $\langle L \rangle$  and the standard deviation  $\sigma_L$  of F1-5 are provided as insets, calculated with FiberApp<sup>6</sup>.



FIG. S4. Comparison of the scattered intensity of the sample S with distinct backgrounds B. Sample F1 (see Fig. S3 is employed. (a) Sample at rest in quartz capillary with 1000  $\mu$ m of diameter with exposure time of 0.1 s. (b) Sample under flow in a PI/NOA/PI chip with 250  $\mu$ m of thickness with exposure time of 0.5 s. (c) Sample under flow in a glass/NOA/PI chip with 250  $\mu$ m of thickness with exposure time of 0.5 s. (d) Corrected intensity of samples with three distinct backgrounds, normalized by the exposure times. The range of scattering vectors  $\Delta q = 0.37 - 1.05 \text{ nm}^{-1}$  indicates the region of interest for the azimuthal integration, which includes signals scattered by dimensions between  $\Delta d = 6-17 \text{ nm}$ . The flow rate was maintained at 5  $\mu$ l/min with a syringe pump.