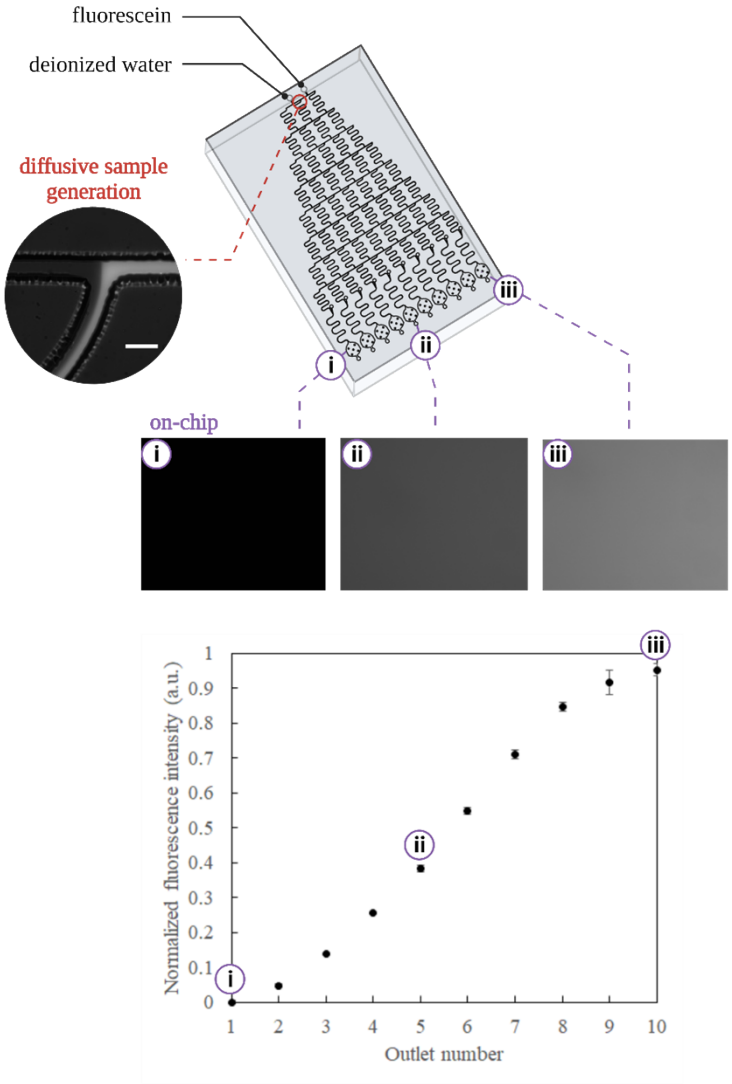
Supplementary discussion

Platform operation

The platform can generate an array of concentrations of fluorescein, as seen in **SI1**.

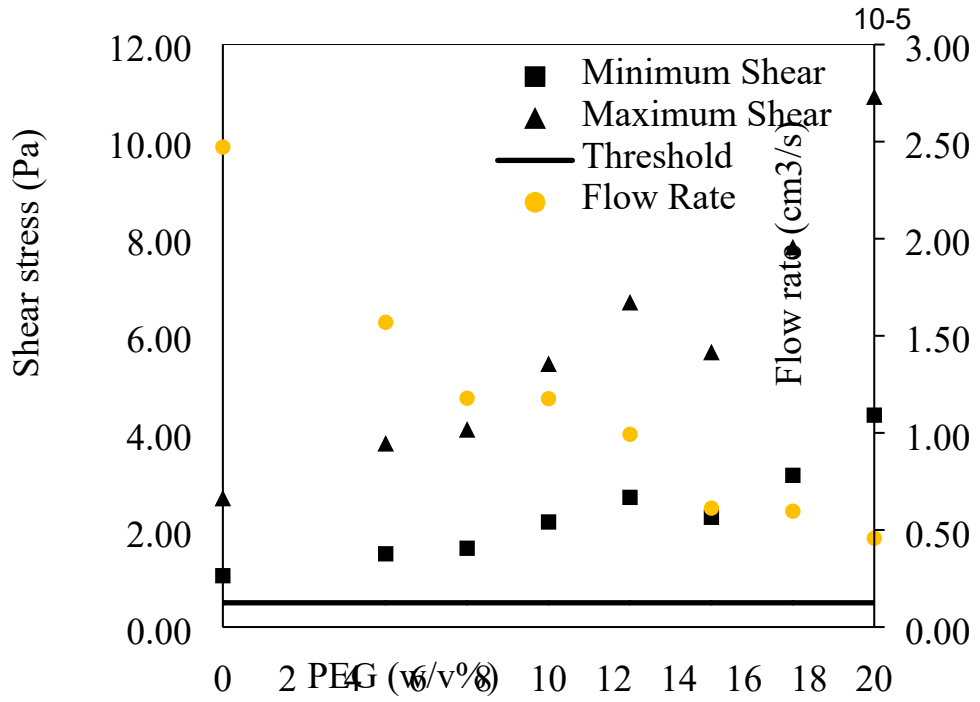


SI1. Generation of a sample array with varying fluorescein concentrations using a single-layer diffusive gradient micromixer device mounted on glass. An array of samples is generated from inlet solutions of deionized water and fluorescein. Three representative fluorescence micrographs are shown of the reservoirs with specific fluorescence intensities and corresponding concentrations of fluorescein. The merged brightfield and fluorescence image shows the junction at which the deionized water and fluorescein solutions meet and begin mixing (scale bar: 40 µm). A plot of normalized fluorescence intensity and corresponding reservoirs describes a linear distribution of concentrations in the reservoirs.

SI2 describes the shear experienced in the system as determined by micro-rheology and Poiseuille's flow. Even though shear does change with respect to PEG concentration and flow rate, all samples in a single experiment (same PEG concentration) and all samples in a single phase diagram are subject to shear above the phase separation threshold. Since both the maximum and minimum shear stresses are above the threshold, our experimental parameters are always within the domain where phase transitions are mediated by shear.

We minimize differences in viscosity in the micromixer by introducing the highly viscous trigger, PEG, only after the sample array is generated.

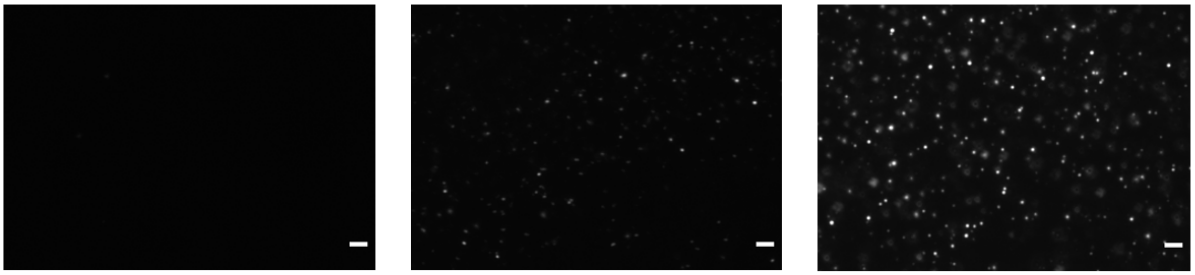
Although we must normalize the concentrations for PEG flow rates in our platform due to the use of pressure pumps, this calculation can be avoided in future implementations of the platform by using volumetric flow rate-controlled pumps.



SI2. Shear stress in the microfluidic system. Shear stress greater than 0.5 Pa (black line) denotes the domain where phase transitions can be mediated by shear stress. All shear stresses (minimum shear, black squares; maximum shear, black triangles) used in our experiments are greater than the threshold above which shear can induce phase separation of liquid-liquid phase separating proteins at concentrations and conditions different than non-shearing conditions. 10 samples are used for each data point.

Bulk assays for Rv1747

Bulk assays are performed to determine an approximate range of protein and PEG concentrations to use in microfluidic experiments, as seen in **SI3**.



SI3. Liquid-liquid phase separation in bulk assays. Fluorescence microscopy images of increasing concentration of $Rv1747^{1-310}$ in 12 w/v % PEG in well-like PDMS devices mounted on #1.5 glass (Ted Pella). The phase separation threshold with respect to protein concentration is between 0.046mg/ml and 0.062mg/ml at this particular concentration of PEG. Samples are imaged at x10 magnification (scale bar: 20 μm).

Time scales

We approximate the diffusion coefficient using the Stokes-Einstein relation, as described below in Equation 3:

$$D = \frac{k_b T}{6\pi\eta r_h} \quad [3],$$

where k_b is Boltzmann's constant, T is the temperature, η is the dynamic viscosity, and r_h is the hydrodynamic radius of the protein with respect to molecular weight. η is determined using viscometer measurements. The resulting approximate diffusion coefficient of the protein has an order-of-magnitude of $10^{-7}\frac{cm^2}{s}$.

We then approximate the characteristic time as in Equation 4:

$$t_l \approx \frac{\Delta L_l^{\,2}}{2D} \quad [4],$$

where t_l is the characteristic diffusion time scale, ΔL_l is the distance that protein would have to diffuse to reach an undesired channel (lateral distance between an inlet and the midpoint of a branch, 1.47 mm), and D is the diffusion coefficient.

Following microrheology experiments to determine velocity, we calculate the forward characteristic diffusion time scale using the relation described by Equation 5:

$$t_f \propto \frac{L_f}{u} \quad [5],$$

where t_f is the characteristic advection time scale of particles carried by the flow, L_f is the characteristic forward length (length of inlet channel, 780μm), u is the velocity of the flow."

The resulting lateral time scale is four orders-of-magnitude greater than the time scale of forward flow. This means that the experimental design minimized the effects of lateral diffusion into undesired channels. However, the ratio of lateral-to-forward time scales is not infinite. While the effect of lateral diffusion may be small (0.01% of forward time scale), it is not zero, and therefore there may be small amounts of protein that laterally diffused to end up in the lowest concentration outlet.