

Electronic Supplementary Material

Hydrophobin: fluorosurfactant-like properties without fluorine

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1. Measurement of HFBI isoelectric point

The isoelectric point of HFBI was measured experimentally with a Nano-ZS zetasizer (Malvern instruments, UK) by performing titrations with 0.01M and 0.1M NaOH on 0.10 mg ml⁻¹ protein solutions in mQ water.

The measurements were highly reproducible, and the experimental pI obtained as an average of three different measurements was 6.08. For comparison, the theoretical pI calculated with Expsy software was 5.7.

In **Fig. S1** is reported a typical experimental titration curve.

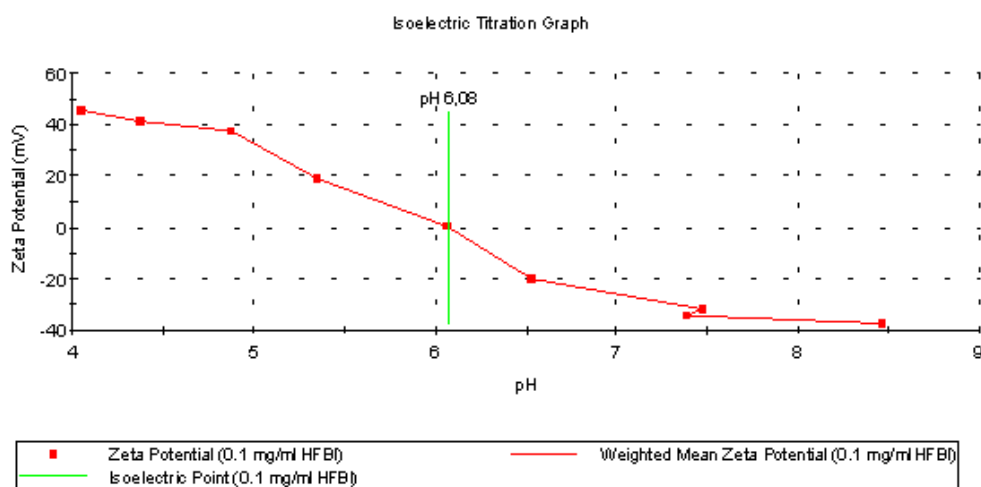


Fig. S1. Titration curve for the measurement of HFBI pI. The final result was obtained as an average of three different measurements.

2. Tensiometric measurements at varying pH values

Tensiometric measurements at several pH values were performed by the pendant drop method. A 15 μl droplet of GSV90 was injected into 900 μl of freshly prepared 0.10 mg ml^{-1} HFBI solutions in 20 mM aqueous buffers (citrate buffer, pH 4; sodium acetate buffer, pH 5.5; phosphate buffer, pH 7; boric acid buffer, pH 10) in a quartz cuvette, and the measurement was started immediately. Droplet shape fitting was performed by the Young–Laplace method. The curves reported in **Fig. S2** illustrate the interface tension vs. time.

The results are very similar in all cases.

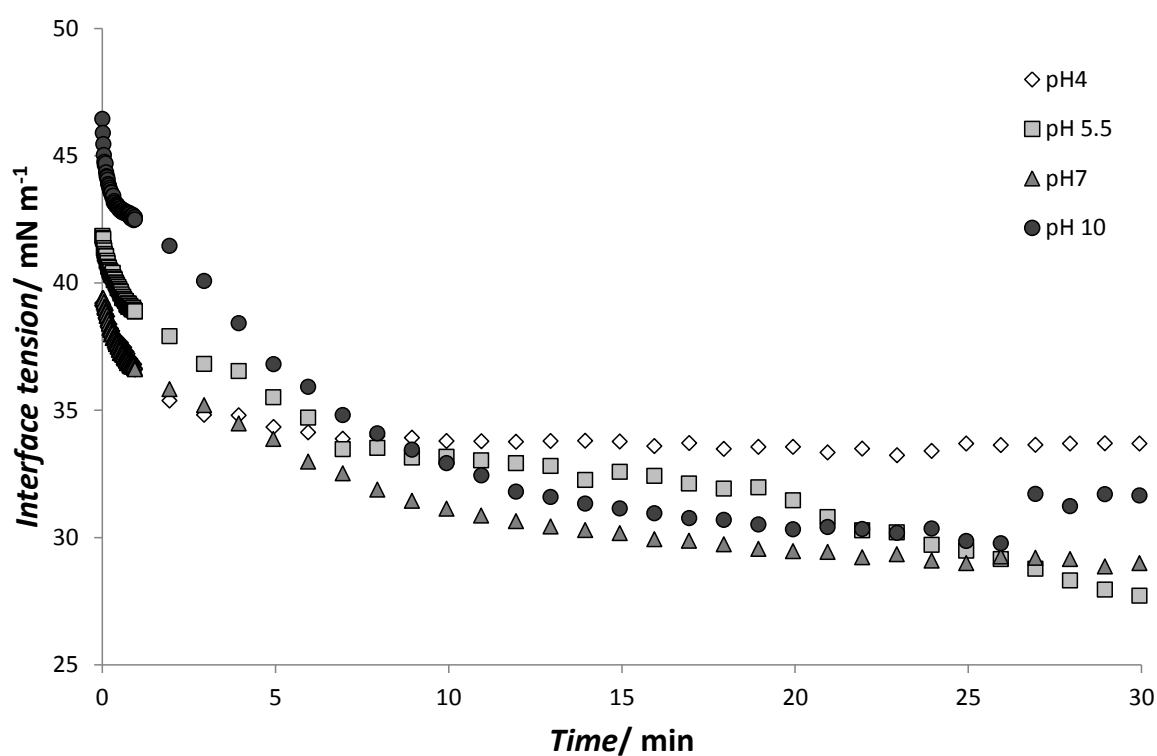


Fig. S2. Plot of interface tension vs. time for GSV90 droplets immersed in buffered aqueous solutions of HFBI (0.10 mg/ml) at different pH values.

3. Detailed description of the microfluidic circuit

The microreactor was a glass-based microdevice with overall dimension of (2.5x6) cm. A B270 glass sheet with photoresist and chrome layers (TELIC, USA) was used as substrate. Uncoated glass was chosen for its chemical inertness, hydrophilicity and well established fabrication methods. The fabrication process consisted of UV exposure, heat treatment and HF etching. Two glass plates were etched using specular mask designs and fused by thermal bonding so that an approximately circular cross section of the orifice was obtained. One of the two glass sides was then drilled before bonding, in order to obtain 700 μm diameter holes. HPLC connectors were used to flow liquids inside the reactor.

Droplets were generated from flow focusing geometry, consisting of three channels converging into a main channel via a narrow orifice. In this configuration the dispersed phase, contained in the middle channel, is squeezed by continuous phase flows from the two opposing side channels. Both phases pass through the small orifice that is located downstream of the three channels. Finally, the stream of the dispersed phase becomes narrow and breaks into droplets. The droplet size is determined by the flow rates of the two phases and by the flow rate ratio, in addition to the channel geometries and the viscosities of the two phases.

The reactor geometry consisted of several parts:

- two inlets, one for water (continuous phase) and one for oil, FC-70, (dispersed phase) injection (width $w_1 = 420\mu\text{m}$, depth $d_1 = 100\mu\text{m}$). The water channel had a bifurcation which converged at the nozzle and was responsible of shear forces and generation of oil droplets;
- a classical flow focusing nozzle ($w_2 = 100\mu\text{m}$, $d_2 = 80\mu\text{m}$) enabling the production of oil droplets in a continuous water phase;
- a serpentine channel ($w_3 = 420\mu\text{m}$, $d_3 = 100\mu\text{m}$ and length $L_3 \sim 15\text{cm}$);
- a collecting chamber ($w_4 = 2\text{cm}$, $d_4 = 100\mu\text{m}$ and $L_4 = 1.6\text{cm}$), for droplet coalescence evaluation.

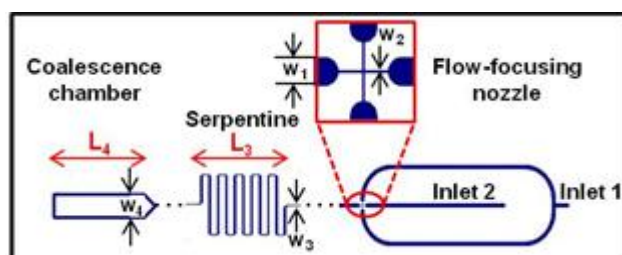


Fig. S3. Schematic representation of the flow focusing microfluidic circuit employed.

Further images of the microfluidic circuit in the nozzle area and film formation on the walls of the circuit are reported below.

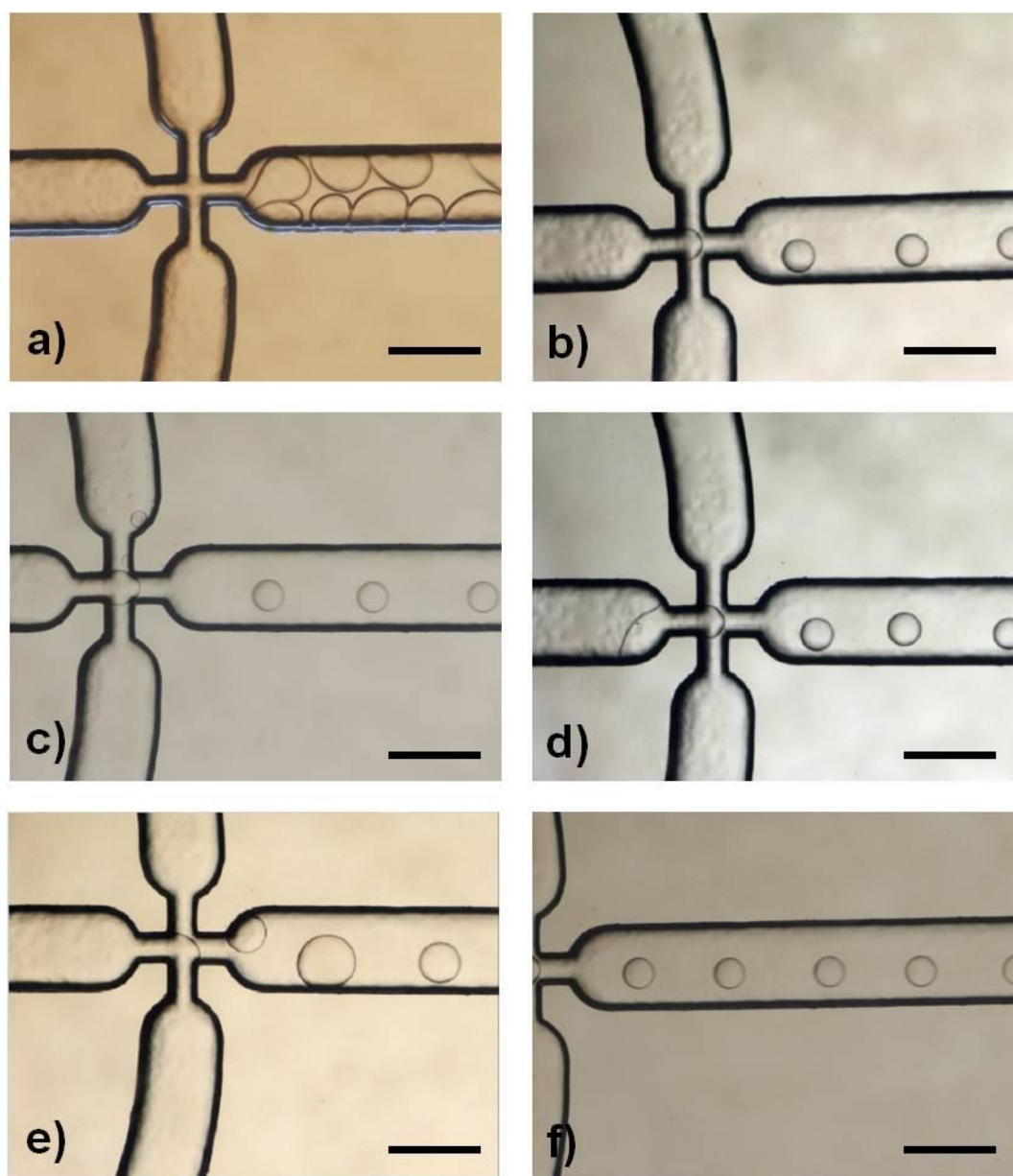


Fig. S4. Optical images at the nozzle for H₂O/FC-70 at a) pH 10.4 and b) pH 13.0 without surfactant; for HFBI in H₂O/FC-70 at c) pH 10.4 and d) pH 13.0; for Zonyl FSN-100 in H₂O/FC-70 at e) pH 7.0 and f) pH 13.0. The scale bar in the figures is 350 μ m.

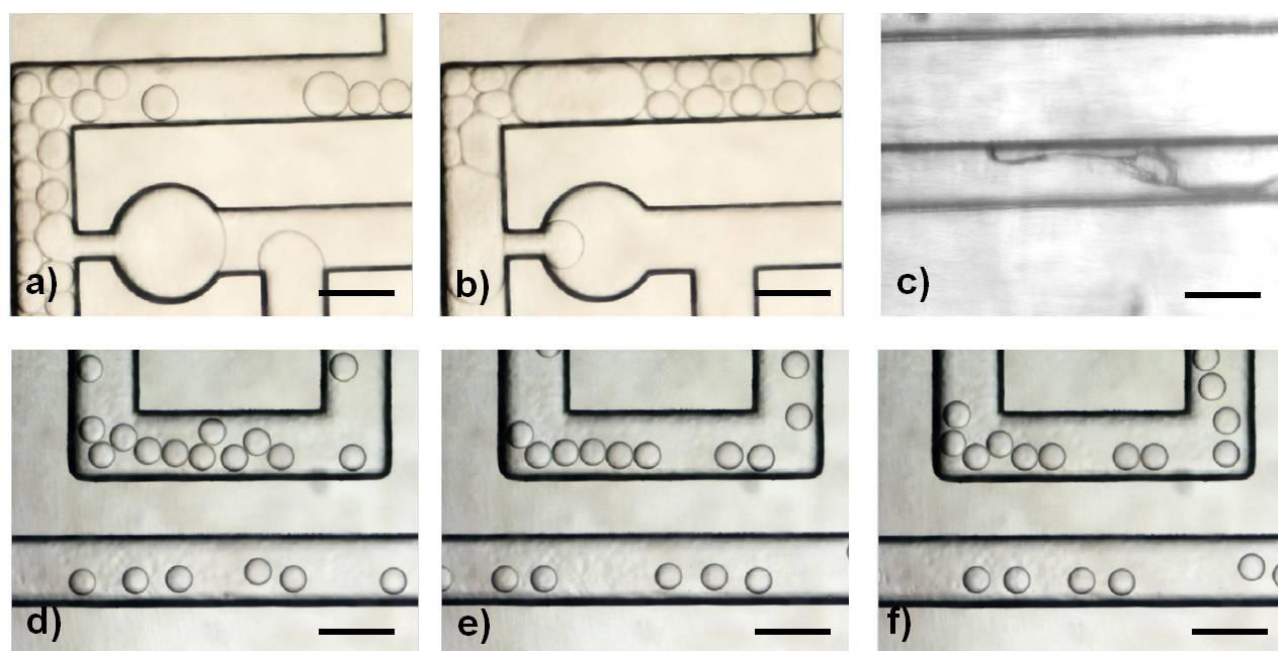


Fig. S5 Optical images in the serpentine region for HFBI in H₂O/FC-70 at pH 10.4 a) frame 1 and b) frame 2 (acquired 0.13 s after frame 1); optical images of the serpentine region c) formation of HFBI film on microchannel walls at pH 10.4. Optical images in the serpentine region for HFBI in H₂O/FC-70 at pH 13.0: d) frame 1, e) frame 2 (acquired 0.13 s after frame 1), and f) frame 3 (acquired 0.13 s after frame 2). The scale bar in the figures is 350 μ m.