

SUPPORTING INFORMATION FOR

Controlling non-specific protein adsorption in a plug-based microfluidic system by controlling interfacial chemistry using fluoruous-phase surfactants.

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ABSTRACT: This supporting information section contains the details of the R_f OEG extraction and the four figures that were mentioned within the primary article.

R_f -OEG Extraction.

In a typical preparation, Zonyl FSO-100 (2 mL) was shaken vigorously in FC-3283 (10 mL). The resulting heterogenous mixture was filtered through a tightly packed cotton plug. The filtrate was extracted once with water (10 mL), yielding an emulsion. The emulsion was centrifuged to separate the fluoruous phase. The fluoruous phase was decanted and stored at 2° C in the dark for later use. Zonyl FSO-100 (DuPont, Wilmington, DE) is a mixture of perfluorinated alkanes capped with oligoethylene

glycol groups having the general structure $\text{CF}_3(\text{CF}_2)_m(\text{CH}_2\text{CH}_2\text{O})_n\text{H}$. Electrospray mass spectrometry showed that the aqueous extraction removed water-soluble species with large values of n . Furthermore, the fluoruous extract contained a mixture of species with $m = 5, 7, \text{ and } 9$, and 11 with $n = 3, 4, \text{ and } 5$. The average molecular weight of $\text{R}_f\text{-OEG}$ was determined by MS to be 557 g mol^{-1} . $^1\text{H NMR}$ was used to determine the concentration of FSO in fluoruous solution. Fluoruous FSO extract ($200 \mu\text{L}$), $1H,1H$ -perfluorooctylamine ($20 \mu\text{L}$, Lancaster Synthesis, Lancashire, UK), and FC-3283 ($1000 \mu\text{L}$) were combined in an NMR tube. Integration of the peaks gave the concentration of $\text{R}_f\text{-OEG}$ in the original fluoruous extract as $\sim 2 \text{ mg mL}^{-1}$. We could not exclude contamination of the surfactant by the corresponding $1H,1H,2H,2H$ -perfluoroalcohols (which should be less surface active than $\text{R}_f\text{-OEG}$ and should not affect surface properties significantly). The extract was further diluted with FC-3283 prior to use in a 1:4 (v/v) ratio, so the experimental concentration of $\text{R}_f\text{-OEG}$ in fluorocarbon oil was $\sim 0.5 \text{ mg mL}^{-1}$. To check for remaining aqueous solubility of FSO, an aliquot of the original filtrate was extracted twice. The $^1\text{H NMR}$ spectrum gave integrations that were identical to those observed in a singly extracted FSO solution, indicating that not a significant amount of the surfactant was removed by the second aqueous extraction.

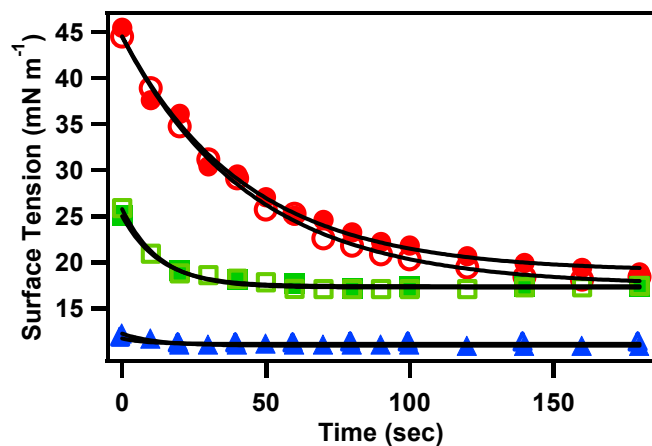
Figure S-1 shows drop tensiometry measurements for alkaline phosphatase at interfaces that presented one of the three following surfactants: $\text{R}_f\text{-COOH}$, $\text{R}_f\text{-CH}_2\text{CH}_2\text{OH}$, or $\text{R}_f\text{-OEG}$.

Figure S-2 shows the Selwyn's plots for alkaline phosphatase kinetics measured within a microfluidic device for plugs formed with one of the following three surfactants: $\text{R}_f\text{-COOH}$, $\text{R}_f\text{-CH}_2\text{CH}_2\text{OH}$, or $\text{R}_f\text{-OEG}$.

Figure S-3 shows tensiometry data for variable concentrations of fibrinogen at an interface that presents the $\text{R}_f\text{-COOH}$ surfactant.

Figure S-4 shows tensiometry data for 1 mg mL^{-1} fibrinogen at a pure fluorocarbon oil interface.

Figure S-1. Graphs of surface tension versus time for AP at interfaces that presented one of the following surfactants: R_f-COOH (●), R_f-CH₂CH₂OH (▲) and R_f-OEG (■). Open symbols indicate (Buffer)/(Surfactant+FC) control droplets, closed symbols indicate (Buffer+AP)/(Surfactant+FC) test droplets. For all surfactants, the surface tension of the test droplet matched the surface tension of the control droplet. Therefore, we concluded that AP does not adsorb at interfaces that presented any one of the three surfactants. AP concentration was 10 μg mL⁻¹ in 1M DEA buffer.



Selwyn's test is performed by taking the kinetic data for $[P]$ versus t (Figure S-2 a,c) and re-plotting it as $[P]$ versus $[E]_0 \times t$ (Figure S-2 b,d) at the same substrate concentration, where $[P]$ is the time-dependent product concentration, $[E]_0$ is the initial enzyme concentration and t is time. If there is no enzyme denaturation, then all the kinetic curves within the $[P]$ versus $[E]_0 \times t$ plot should superimpose on one another. Data were analyzed for three substrate concentrations. Enzyme concentration was varied at each substrate concentration. For AP, Selwyn plots indicated that all kinetic curves for R_f-COOH did not superimpose with the fit curves. However, there was only a slight deviation for both $w = 150 \mu\text{m}$ (Figure S-2b) and $w = 50 \mu\text{m}$ (Figure S-2d).

Figure S-2. Selwyn's test for adsorption with alkaline phosphatase (AP). AP kinetics was measured within microfluidic devices for $w = 150 \mu\text{m}$ (a, b) and $w = 50 \mu\text{m}$ (c, d) for three different surfactants: R_f-COOH (●), R_f-CH₂CH₂OH (▲) and R_f-OEG (■). (a, c) Kinetics was measured for 2 μM fluorescein diphosphate (FDP) in DEA buffer at pH = 10 and AP concentration was varied from 0.05 μM , 0.1 μM to 0.2 μM . Black dashed lines show fluorimeter data at the same concentrations and solid black curves represent expected kinetic curves for fully active AP, where $k_{\text{cat}}/K_M = 0.75 \mu\text{M}^{-1} \text{s}^{-1}$. In (b, d), Selwyn plots are shown, where kinetic data from (a, c) were plotted for product concentration versus $[E]_0 \times t$ for the same substrate concentration, where $[E]_0$ is the initial enzyme concentration and t is time. In Selwyn plots, all kinetic curves for (Buffer+AP)/(R_f-COOH+FC) do not superimpose with the black fit curves. Kinetic curves for (Buffer+AP)/(R_f-CH₂CHOH+FC) superimpose with the black fit curves only when $w = 50 \mu\text{m}$ but not when $w = 150 \mu\text{m}$. However, all kinetic curves for (Buffer+AP)/(R_f-OEG+FC) superimpose with the black fit curves.

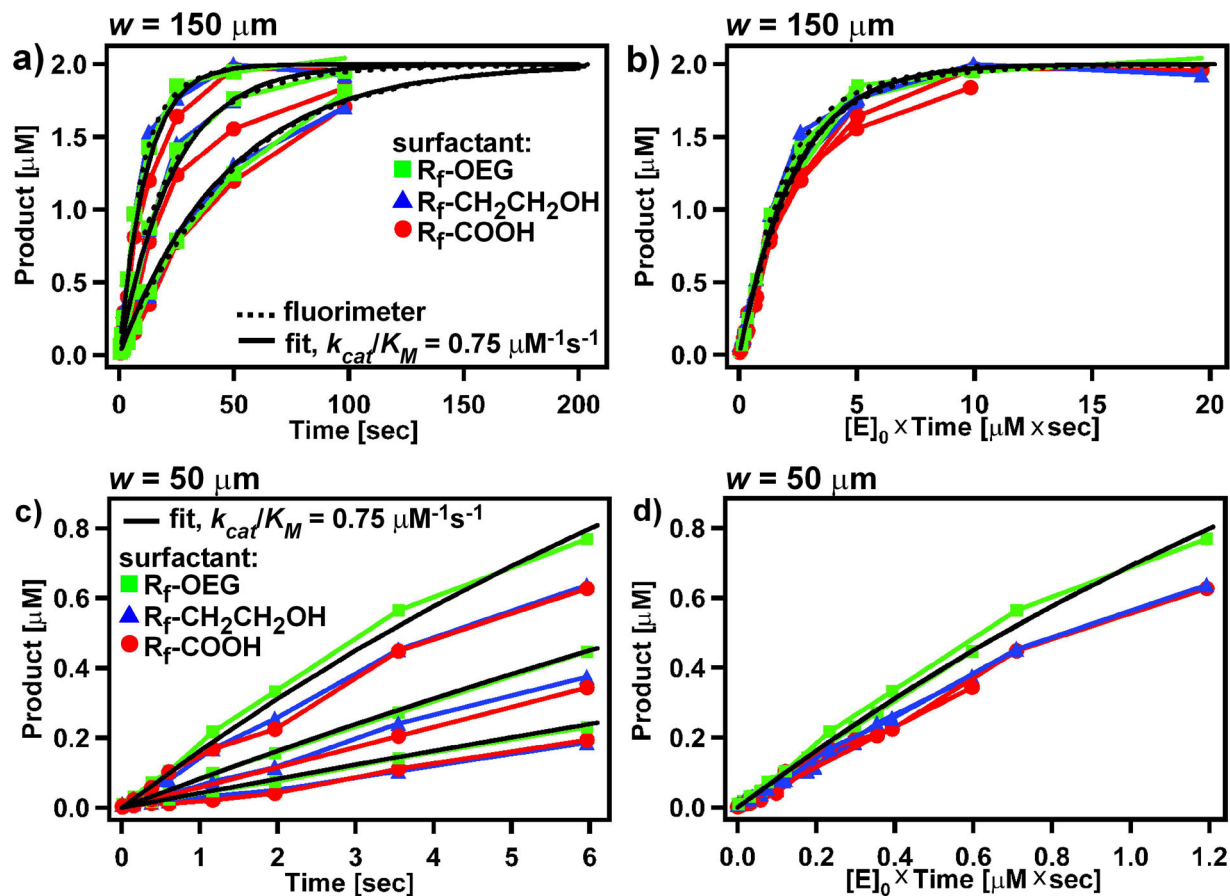


Figure S-3. Variable concentrations of fibrinogen adsorbing at an interface presenting the R_f -COOH surfactant. More concentrated fibrinogen solutions absorb faster than less concentrated solutions, but all reach the same equilibrium surface tension.

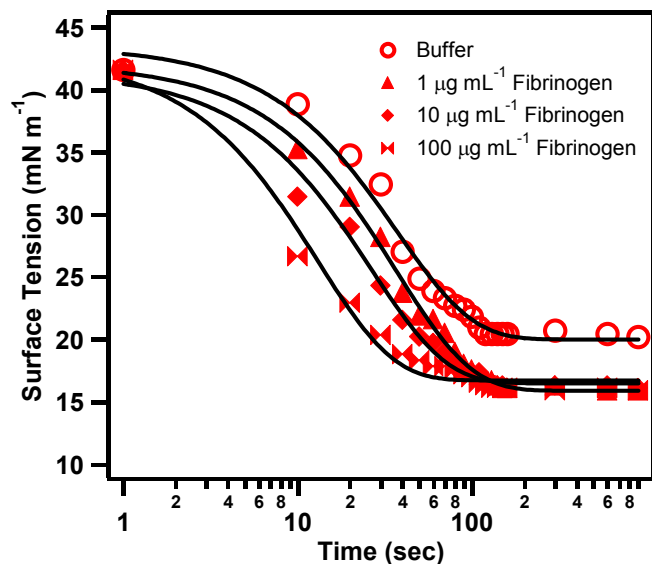


Figure S-4. Adsorption of 1 mg mL⁻¹ fibrinogen in PBS buffer to a pure fluorocarbon oil interface.

