Autonomous Directional Motion of Actin-Containing Cell-Sized Droplets

Barbara Haller, Kevin Jahnke, Marian Weiss, Kerstin Göpfrich,* Ilia Platzman,* and Joachim Pius Spatz*

Cell motility is potentially the most apparent distinction of living matter, serving an essential purpose in single cells and multicellular organisms alike. Thus, the bottom-up reconstitution of autonomous motion of cell-sized compartments remains an exciting but challenging goal. Herein, actin-driven Marangoni flows are engineered to generate rotational and translational motility of surfactant-stabilized emulsion droplets. The interaction between actin filaments and the negatively charged block-copolymer Krytox is identified as the driving force for Marangoni flows at the droplet interface. Tuning the actin–Krytox interplay, sustained autonomous unidirectional droplet rotation with 1.7 rot h⁻¹ is achieved. Ultimately, this rotational motion is transformed into a translational rolling motion by introducing interactions between the droplets and the surface of the observation chamber. Accordingly, translational motility of actin-containing droplets at velocities of 0.061 ± 0.014 μm s⁻¹ is reported herein and an overall displacement of several hundreds of micrometers within 30 min is observed. These self-propelled systems with biologically active molecules demonstrate how motility could be implemented for synthetic cells.

1. Introduction

Many processes in living cells, including chemotaxis in unicellular organisms or embryonic development, immune response, tissue formation, and wound healing, are governed by motility. Mechanisms of motion, such as flagella propulsion in bacteria and spermatozoa or cytoskeletal forces in migrating cells, are widely studied, but the extensive complexity of these mechanisms has hindered a full understanding. In addition to intracellular contractile and pushing cytoskeletal forces cell movement is governed by cell–cell and cell–extracellular matrix interactions. Moreover, cell membrane flows have an additional effect on cellular locomotion. Despite being less discussed in the literature, membrane flows allow for membrane surface enlargement in the front of the cell due to locomotion-mediated protrusions. The additional membrane surface can be provided for example by exocytosis of internal vesicles at the front of the cell body and the accompanying endocytosis at the cell rear. These processes generate a lateral flow of lipids in the membrane from the front to the rear. Thus, it is conceivable that membrane access and release together with the accompanied flows are directly involved in the propulsion mechanism. Supporting this hypothesis, a theoretical model showed that tangential traveling surface waves could cause a translational movement in spherical microsized objects. In any case, the exact contribution of these membrane flows is not well understood and requires further investigations. Bottom-up synthetic biology offers a possible route toward a systematic understanding of membrane flows in the context of cellular locomotion. By generating an autonomous motion in a synthetic system consisting of minimal biological building blocks, one could draw conclusions on how propulsion...
evolved in similarly primitive cells at the onset of life and elucidate what role it may play in today’s living systems. Using the toolbox of synthetic biology, several studies have shown the internal dynamics of the cytoskeleton leading to a contraction or deformation of the cell rim.\textsuperscript{8–11} The autonomous motion of the entire minimal cell, however, is more difficult to achieve. For example, autonomous motion was obtained via reconstitution of fluid flows initiated by kinesin activity and collective dynamics of microtubule bundles.\textsuperscript{12–15} In contrast to that, autonomous propulsion of inorganic particles has been achieved by several groups, often exploiting osmotic or phoretic forces, for example, in enzyme-powered Janus particles\textsuperscript{16,17} or surface tension gradients as the Marangoni effect.\textsuperscript{18,19}

The Marangoni effect is governed by local inhomogeneities of the interfacial tension (IFT) at interfaces of emulsions such as water-in-oil droplets. The IFT gradient in turn creates a stress and thus a tangential liquid flow at the interface.\textsuperscript{20} Various factors could be responsible for a nonuniform IFT in water-in-oil droplets, for example, heterogeneous surfactant mixtures, local chemical reactions, and gradients in temperature or surfactant concentrations.\textsuperscript{21}

In this study, by applying bottom-up synthetic biology strategies we provide a systematic investigation of autonomous Marangoni-driven directed motion of a minimal cell system. To achieve that, a minimal actin cytoskeleton was reconstituted within water-in-oil droplets by means of a droplet-based microfluidic technology. Following the formation of actin filaments, we specifically created the interactions of the actin with the surfactants on the droplet’s inner interface, thereby creating a nonuniform IFT leading to fluid flows and rotational motion of the droplet at a velocity of up to $1.7 \pm 0.5 \text{ rot h}^{-1}$. Moreover, by modifying the interaction of surfactants with a glass interface we show that translational motion of actin-containing droplets can be achieved. Notably, unlike the motion of enzyme-accelerated Janus particles, the motion demonstrated here is mainly a spontaneous directional motion.\textsuperscript{22} Importantly, this study shows how biologically relevant elements of the cellular motility system, namely, actin filaments, can generate autonomous directional motion based on the Marangoni effect.

2. Results and Discussion

Migration of natural cells is mainly induced by the forces applied on the cell rim due to cytoskeletal dynamics. In the process of locomotion, actin-mediated protrusions on the cellular membrane induce lipid membrane flow to compensate for the growth of the membrane surface area. Inspired to reconstitute a minimal locomotion system, we set out to establish an actin-containing synthetic cell model system that will be able to generate autonomous membrane flow-mediated directed motion. Due to mechanical and chemical instabilities of the cell-sized lipid-based vesicles,\textsuperscript{23} we decided to use surfactant-stabilized water-in-oil droplets as a stable compartment system. Moreover, instead of a cytoskeletal dynamics mediated membrane flow we aimed to apply actin filaments to generate autonomous directional surfactant membrane motion based on the physicochemical Marangoni effect. To generate mass transfer along the surfactant layer at the oil–water interface, a gradient of the surface tension must be maintained over time. Therefore, a continuous interaction between the actin filaments and the droplet interface is required to preserve the IFT gradient. Figure 1 shows a schematic representation of the envisioned concept for the Marangoni-driven motion of the actin-containing synthetic cell. To create charge-mediated interactions between actin filaments and the droplets’ inner interface, the water-in-oil droplets are stabilized by a mixture of two types of surfactants exhibiting distinct interfacial properties: an uncharged triblock surfactant and cationic Krytox clusters.

![Figure 1](image-url) Figure 1. Schematic illustration of the physicochemical processes driving the actin-induced Marangoni flow within water-in-oil droplets. A surfactant-stabilized water-in-oil droplet containing actin filaments is shown in the center of the figure. The insets illustrate actin–surfactant interactions, including A) Mg\textsuperscript{2+} ions-mediated interactions of the negatively charged actin filaments and Krytox at the droplet periphery; B) the local formation of Krytox clusters and a corresponding change in IFT; C) the rearrangement of the diffusive surfactant layer due to IFT gradients; D) micellar exclusion of actin monomers due to surfactant desorption from the surfactant layer.
copolymers perfluoropolyether-poly(ethylene glycol)-perfluoropolyether (PEG-based) fluoro surfactant and a negatively charged perfluoropolyether (PFPE) carboxylic acid fluorosurfactant (Krytox). The distribution of negatively charged surfactants at the droplet interface attract Mg$^{2+}$ ions\cite{24} that, in turn, induce electrostatic interactions with the negatively charged actin filaments. In a natural cell, the linkage of actin to the cellular membrane is mediated by numerous components, including transmembrane proteins such as integrin and a variety of anchor proteins.\cite{2} Therefore, by using the electrostatic interaction between the encapsulated actin and Krytox as shown in Figure 1A we bypass the complexity of actin–membrane interactions in natural cells. The local interaction of actin filaments with the Krytox surfactant leads to Krytox clusters at the interaction points, creating IFT gradients across the interface (Figure 1B). As a response to the interfacial stresses, the diffusive surfactant layer\cite{25} rearranges laterally (Figure 1C). In addition to lateral mobility, surfactants also absorb to and desorb from the droplet periphery, leading to micellar exclusion of actin monomers (Figure 1D). Note that depolymerization and denaturation of actin filaments at the droplet interface can be attributed to a local Krytox-mediated acidic environment. These processes are crucial for the creation of a continuous liquid flow due to the formation of new actin–Krytox interactions that in turn maintain a distinct IFT gradient at these interaction points.

### 2.1. Interaction of Actin with Krytox

To test our concept, we initially set out to establish a connection between actin and the droplet interface. For that purpose, we first used droplet-based microfluidics to encapsulate 10 μm actin filaments (containing 1% Alexa568- or Alexa633-labelled actin) in actin polymerization buffer (see Experimental Section) into water-in-oil droplets (see Figure S1, Supporting Information, for the layout of the microfluidic device). The droplets were stabilized by a mixture of 1.4 wt% uncharged PEG-based fluorosurfactant and different concentrations of negatively charged Krytox (0.04, 0.54, 1.04, and 5.04 mM). Note, pure PEG-based surfactants with no traces of Krytox cannot be achieved due to the synthesis and purification procedures. Therefore, the commercially available surfactants also contain Krytox traces.\cite{24} The fluorescence signal of actin filaments within the droplets was observed by confocal fluorescence microscopy. As visible in Figure 2A, nearly no actin filaments are colocalized with the droplet interface in the presence of only 0.04 mM Krytox (note that the 0.04 mM Krytox is the minimal achievable concentration;\cite{24} no Krytox was added here). However, at the higher Krytox concentrations a clear actin fluorescence ring at the droplet interface was observed. Moreover, as visible in the confocal images, the fluorescence signal of actin in the droplet interior (excluding the periphery) is decreasing with increasing Krytox concentrations. Eventually, actin loses its fibrous structure at elevated Krytox concentrations (see Video S1, Supporting Information). The loss of fibrous structures at high Krytox concentrations can be attributed to depolymerization and denaturation of actin at low pH values.\cite{26} Note that the pH is dropping inside the droplet, because Krytox acts as a proton donor at the droplet interface.\cite{24} The inert PEG-based fluorosurfactant inhibits the interaction between encapsulated actin and the droplet interface while Krytox introduces negative charges at

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**Figure 2.** Interaction between actin and Krytox at the droplet periphery is concentration-dependent. A) Confocal fluorescence images of actin filaments (labeled with 1% Alexa633, $\lambda_{em} = 633$ nm) encapsulated into water-in-oil droplets at different Krytox concentrations in the oil phase. Scale bar: 10 μm. B) Fluorescence intensity of actin at the droplet periphery dependent on the Krytox concentration. With increasing Krytox concentration, the fraction of actin at the droplet periphery increases and reaches a plateau at 3 mM. Error bars correspond to the standard deviation of $n = 8–19$ evaluated droplets. C) IFT of actin buffer with (red) and without actin monomers (blue) at different Krytox concentrations as determined with pendant drop measurements ($n \geq 70$ evaluated droplet shapes). The IFT values are elevated in the presence of actin monomers in the buffer due to the formation of actin–Krytox clusters for all tested Krytox concentrations. The significance was tested using an unpaired Welch’s t-test.
the interface which attract Mg\(^{2+}\) ions from the actin polymerization buffer and, in turn, attract negatively charged actin filaments. Quantitative analysis of the fluorescence intensity at the droplet periphery \(I_{\text{periphery}}\) revealed clear attraction of actin toward the droplet interface at Krytox concentrations above 0.04 mM (Figure 2B).

As an independent confirmation of the Krytox-dependent interaction of actin with the droplet interface, we performed pendant drop measurements (Figure 2C). By the addition of actin monomers to the buffer, the IFT values increased significantly. This confirms the interaction of actin with the Krytox molecules. Note that a higher IFT corresponds to a lower coverage of interfacially active molecules. Thus, the actin–Krytox clusters at the interface form a less dense interface coverage than surfactants in a protein-free surfactant layer. It is important to mention here that the high IFT change as observed for 0.04 mM Krytox can be attributed to the fact that actin monomers were used for this experiment and not polymerized actin fibers, which are less diffusive. Therefore, in the case of experimental conditions, where the actin is polymerized, we do not detect as high IFT as in the case of unpolymerized actin monomers. Note that actin monomers were chosen for this experiment because any traces of magnesium ions in the actin polymerization buffer prevented a stable hanging droplet formation due to electrostatic interactions between Krytox and magnesium at the droplet interface.\(^{[27]}\)

### 2.2. Turnover of Actin–Krytox Interactions

To maintain the motility, which is generated due to local changes in the IFT values on the droplet interface, the system has to be kept out of equilibrium for an extended period of time. To achieve that, local actin–Krytox interactions have to be reformed continuously, meaning that new actin and Krytox molecules have to be constantly recruited to the droplet interface. This condition can be achieved when there is a constant renewal of the actin–Krytox interactions as a result of denaturation and depolymerization and therefore loss of contact and subsequent micellar exclusion of the actin. To test this assumption, we monitored actin-containing droplets (10 \(\mu\)m actin, 1% fluorescently labelled in 10 mM Tris pH7.4, 20 mM MgCl\(_2\)) stabilized by an inert fluorosurfactant and different concentrations of Krytox (0.04, 0.54, and 1.04 mM) over time for 100 min using confocal fluorescence microscopy. The same imaging settings were used for all conditions shown in Figure 3A. In all conditions, the fluorescence intensity inside the droplets dropped over time. Furthermore, the mean fluorescence intensity ratio at the droplet periphery over the droplet interior increased with increasing Krytox concentrations (Figure 3B). Within 100 min, the fluorescence intensity ratio \(I_{\text{periphery}}/I_{\text{inner}}\) for 1 mM Krytox increased even by a factor of 2.7. Notably, the choice of the photostable Alexa633 dye and the presence of actin agglomerates in the oil phase indicate that photobleaching only has a minor contribution to the intensity loss. Thus, the plot shown in Figure 3A clearly indicates a correlation of actin loss and the amount of Krytox used to stabilize the droplets, which can be explained by the fact that actin is pulled out of the aqueous droplet interior due to interactions with Krytox. Krytox is assembling at the interface, attracting actin, and desorbing it from the interface by micellar exclusion. Note that the oil reservoir around the droplets is much bigger than the aqueous phase within the droplet, and thus the leakage of actin into the oil phase cannot be monitored by the increase of fluorescence intensity of the oil phase. However, agglomerates of fluorescent actin particles can be detected in the oil phase (see Video S1, Supporting Information), unambiguously confirming that there is a leakage of actin from the droplets. This, in turn, allows for the continuous turnover of actin–Krytox interactions at the interface, maintaining nonequilibrium interfacial stresses which result in Marangoni flows over a prolonged time period until the actin inside the droplet is largely depleted.

### 2.3. Rotational Motility of Droplets

Time-lapse fluorescence imaging experiments were performed to analyze the conditions required for actin-containing droplet motility. As shown in Figure 4A and in Video S2, Supporting Information, we observed a directed rotational motion of the
droplets at the lowest Krytox concentration (0.04 mM). Rotation, instead of translational movement, is caused because the droplets experience insufficient friction at the upper cover slide of the observation chamber. Remarkably, around 96% (n = 82) of the examined droplets exhibit a rotational motion over several hours with rotational velocity of $1.7 \pm 0.5 \text{ rot h}^{-1}$. In particular, the actin-containing droplets have a constant and a very similar angular velocity, but the axis of rotation of the droplets is random in x-, y-, and z-direction. The high rotational velocity we obtain is remarkable given that the rotational motion is around 28 times faster than the rotational diffusion coefficient (see Supporting Note 1, Supporting Information). In comparison, the enhanced diffusion observed for Janus particles exceeds their diffusion coefficient only by a factor of around 1.5.\[22] Surprisingly, when the Krytox concentration in the oil phase was higher than 0.04 mM no droplet rotation or only minor movements ($<0.5 \text{ rot h}^{-1}$) were observed (n = 57) (Figure 4B and Video S3 and S4, Supporting Information). The absence of motility at high Krytox-concentrations can be attributed to fast actin filaments depolymerization and denaturation. Due to depolymerization, actin monomers create interactions with the droplet interface in a homogeneous manner with no localized change in IFT. Therefore, the Marangoni flows are inhibited. To prevent depolymerization of actin filaments at elevated Krytox concentrations, the filaments must be stabilized. Toward this end, we bundled the filaments by adding 0.4 wt% methylcellulose (MC) to the actin polymerization buffer.[28,29] Availability of MC in the actin polymerization buffer leads to a strong bundling of actin fibers due to the depletion effect, as can be observed in Figure 4C.[30] As expected, under these conditions, we observed a rotational motility of the droplets also in the presence of 0.54 mM Krytox (for 90% of the droplets) and even in the presence of 1.04 mM Krytox (for 72% of the droplets) (Figure 4D).

For a final confirmation that Krytox–actin interactions are the driving force for the autonomous movement of the droplets, we completely inhibited the interaction between the droplet interface and the encapsulated actin. This was achieved by using an outer fluorinated oil phase in which Krytox could not be dissolved (see Experimental Section). Not surprisingly, no rotation of the actin-containing droplets and no decrease in actin fluorescence intensity could be observed in this case (Video S5, Supporting Information). Taking the presented evidence together, we have proven that the model shown in Figure 1 is indeed explaining the observed motion. We can conclude that Marangoni flows can cause motion in minimal cells, fueled by autonomous transport of biologically relevant molecules.

2.4. Translational Motility of Droplets

Ultimately, we set out to engineer translational motility of actin-containing droplets induced by Marangoni flow. From the previous experiments, we know that actin-containing droplets exhibit a rotational motion in an observation chamber assembled from untreated glass cover slides, as shown in Figure 5A. Due to the higher density of the fluorinated oil compared to the aqueous phase, buoyancy forces act on the droplets and bring them in contact with the upper cover slide. We hypothesize that the fact that we observe a rotational motion can be attributed to the absence of friction forces between the droplets and the hydrophilic glass surface. To test this hypothesis, and to induce translational motility, we used the fluorinated glass cover slide to generate friction between a actin-containing droplets and the cover slide, as shown in Figure 5B. As expected, we observed a translational rolling motion of actin-containing droplets in contact with the fluorinated cover slide. The actin-containing droplets, which experience Marangoni flows, cover a distance of several hundreds of micrometers, whereas droplets filled only with ultrapure (Milli-Q) water in the same observation chamber only show insignificant random movement (see Figure S2, Supporting Information). Note that we used actin mixed with 0.4% MC to enhance actin filament stability and therefore increase the observation period. Importantly, we proved that the rolling motion is solely induced through actin-mediated
Marangoni flow by mixing MQ- and actin-containing droplets within the same observation chamber. We analyzed their respective velocities for n = 18 droplets and found that actin-containing droplets move with an average velocity of 0.061 ± 0.014 μm s⁻¹, whereas MQ-containing droplets are almost stationary with 0.010 ± 0.003 μm s⁻¹ (see Figure 5C).

Thus, the velocity of actin-containing droplets exceeds that of MQ-containing droplets by a factor of six. Moreover, from the observations it is evident that the movement of MQ droplets is often caused simply by interactions with passing actin-containing droplets. Figure 5D,E shows the displacement of individual droplets over time. Even the fastest moving MQ droplets cover a maximum distance of 58 μm, whereas the fastest actin-containing droplets move more than 400 μm. In addition, it shows that some actin droplets stick to a rotational motion in presence of a fluorinated surface, whereas others alternate between a rolling and a rotational motion. This could be potentially attributed to imperfections of the fluorinization of the surface. Note that large droplets with diameters of 60 μm or bigger do not exhibit any rotational or rolling motion because they experience too much friction with the coverslip. To exclude the effect of droplet size on the motility, we set the diameter to ≈40 μm.

To compare rolling and rotation, we calculated the corresponding rotational frequency from the average translational displacement of actin-containing droplets. This yields a frequency of $\omega = \frac{\nu_{\text{trans}}}{2\pi r} = 1.75 \pm 0.40$ rot h⁻¹ for a droplet radius of 20 μm. This is in excellent agreement with the presented values for rotating droplets in contact with an untreated cover slide, which we found to be $1.7 \pm 0.5$ rot h⁻¹.

3. Conclusion

In this study, we demonstrated a method to generate directed autonomous motion of cell-sized actin-containing droplets mediated by Marangoni flows. Key elements are 1) a locally restricted Mg²⁺-mediated interaction of the negatively charged actin filaments with the negatively charged fluorosurfactant (Krytox) at the droplet interface, 2) the resulting local change in IFT, and 3) the maintenance of this out-of-equilibrium state by constant assembly and disassembly of Krytox–actin interactions along with the transport of actin across the interface. These conditions lead to Marangoni flows at the oil–water boundaries that result in an autonomous rotational motion of the droplets. As water-in-oil droplets are commonly used compartment systems in bottom-up synthetic biology, our study also highlights the physicochemical effects that come into play due to the chemical nature of the surfactants. We show how important it is to be aware of the presence of Krytox in the surfactant mix and how it can even be exploited to achieve desirable functions such as motility. Whereas in other systems enzyme-propelled Janus particles’ motion is in the magnitude of 1.5 times higher than their diffusion coefficient, the here presented rotational speed is significant as it exceeds its rotational diffusion coefficient by the magnitude of ≈28 (rotational diffusion coefficient $D = 0.059$ versus ≈1.7 rot h⁻¹). Moreover, we engineered translational motion of actin-containing droplets by fluorinating the glass interface. This caused a rolling motion of actin-containing droplets over hundreds of micrometers with an average velocity of 0.061 ± 0.014 μm s⁻¹ due to the friction between the droplet and the fluorinated surface. Transforming this into a rotational...
frequencies, we obtain $1.75 \pm 0.40 \text{rot h}^{-1}$ for a droplet radius of 20 $\mu$m, which is in very good agreement with the rotational motion of the droplet and therefore proves that the observed motion is based on actin-induced Marangoni flow.

The presented directed autonomous motion of the water-in-oil droplets could serve as an adequate model system because it mimics membrane flows as described in some primitive living cells. Furthermore, the fatty acid membranes of protocols were more permeable than the phospholipid membranes of today's cells.[13] Therefore, a mechanism of substrate exchange was certainly present. These facts allow for the assumption that the Marangoni flows may have also generated cellular motion in early evolutionary life. Further, it is feasible that the here presented system could be transferred into a physiological more relevant water-in-water system by using an adequate surfactant mixture that allows for a double-layer structure. Self-propelled systems with biologically active molecules are not only a milestone toward the bottom-up assembly of synthetic cells, but also exciting for the development of active drug delivery systems.

4. Experimental Section

Confocal Fluorescence Microscopy: A confocal laser scanning microscope LSM 880, LSM900 (Carl Zeiss AG), or Leica SP5 (Leica Microsystems GmbH) was used for confocal imaging. The pinhole aperture was set to one Airy unit and experiments were performed at room temperature.

The images were acquired using a 20× (Objective Plan-Apochromat 20x/0.8 M27, Carl Zeiss AG) or an 63× oil-immersion objective (HCX PL APO 63×/1.40–0.60; Leica Microsystems GmbH, Germany). For confocal fluorescence imaging, the preformed droplets were sealed in a custom-made observation chamber consisting of two glass cover slides spaced with a double-sided tape. The cover slides were either untreated (Figure 1–4, purchased from Carl Roth) or fluorinated (Figure 5, purchased from Ran Biotechnologies). Images were analyzed and processed with Image (NIH, brightness and contrast adjusted). To analyze the relative intensity of the droplet periphery over the droplet center, $I_{x,y}$ (see Figure 3), a custom-written Python script was used. It first detects the droplet periphery from brightfield images and then defines a 10 pixel wide rim along it to define $I_x$.

Actin Preparation: Actin (purified from acetone powder from New Zealand white rabbit skeletal muscle, based on the method of Pardee and Spudich,[12] modified after Kron et al.[13]) was stored in so-called general actin buffer (GAB) containing 2 $\text{mM} \text{Tris}$/$\text{HCl}$, pH 8, 0.2 $\text{mM} \text{CaCl}_2$, 0.2 $\text{mM} \text{adenosine triphosphate}$ (ATP), 0.005% $\text{NaN}_3$, and 0.2 $\text{mM} \text{dithiothreitol}$ (DTT), at $-80^\circ\text{C}$. The actin monomers were either labeled with methanol-dissolved rhodamine–phalloidin (Biotium), Alexa568, or Alexa633 (Sigma-Aldrich) by mixing 20 $\mu$L actin with 20 $\mu$L double-density actin buffer (50 $\mu$L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.4, 50 $\mu$m KCl, 8 $\mu$m MgCl$_2$, 20 $\mu$m ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), 20 $\mu$m DTT) and 4 $\mu$L 10× actin polymerization buffer (20 $\mu$L Tris-$\text{HCl}$, pH 8, 500 $\mu$m KCl, 20 $\mu$m MgCl$_2$, 10 $\mu$m NaATP). The actin monomers were left on ice to polymerize for 30 min. Subsequently, 10 $\mu$L of rhodamine–phalloidin (10 units) was added to the solution. As an alternative to the rhodamine labeling, 1% custom-made Alexa568 or Alexa633-labeled actin monomers was used. In the indicated cases, 0.4% MC was added to the actin filaments directly before the experiment.

Formation of Surfactant-Stabilized Water-in-Oil Droplets: PDMS-based (Sylgard 184, Dow Corning) microfluidic devices for the formation of water-in-oil droplets were produced and assembled according to previously described protocols.[14] The device layout is shown in Figure S1, Supporting Information. The aqueous phase for droplet production is made of Milli-Q water containing 10 $\mu$m MgCl$_2$, 10 $\mu$m actin, 1× double-density actin buffer (50 mM HEPES, pH 7.4, 50 $\mu$m KCl, 8 $\mu$m MgCl$_2$, 20 $\mu$m EGTA, 20 $\mu$m DTT). The oil phase contained 1.4 wt% of perfluoro-polyether-polyethylene glycol (PFPE-PEG) block-copolymer fluorosurfactants (PEG-based fluorosurfactant, Ran Biotechnologies, Inc.) dissolved in HFE-7500 oil (DuPont) and in the indicated cases mixed with Krytox 157 FS (carboxylic acid-terminated PFPE, DuPont). The fluid pressures were controlled by an Elveflow microfluidic flow control system or syringe pumps (Harvard Apparatus). The fluids were injected into the channels with 1 mL syringes (Omnifit, B.Braun, Germany) connected by a cannula (Sterican 0.4 mm × 20 mm, BL/LB, B.Braun) as well as PTFE tubing (0.4 × 0.9 mm, Bola). To observe the production process, an Axios Vert.A1 (Carl Zeiss AG) inverse microscope was used. As an alternative to the microfluidic formation of droplets, the aqueous phase was layered on top of the oil phase within a microtubule (Eppendorf) and droplet formation was induced by manual shaking as described by Göpfrich et al.[15] The microfluidic approach was used for Figure 2C, 3, and 4, whereas droplets in Figure 2A,B and 5 were produced via the shaking method.

IFT Measurements: A contact angle system OCA (DataPhysics, USA), equipped with a CCD high-speed camera for pendant drop measurement, was used to determine the IFT between MQ or the actin-containing solution and HFE-7500 oil droplets containing PEG-based fluorosurfactants and Krytox. The Young–Laplace equation was chosen to fit the droplet shape to determine the IFT values. To investigate the effect of Krytox on the actin or MQ solution, 0–8 $\mu$m Krytox was added to the oil solution. A stable oil droplet was generated manually into the aqueous solution using a syringe. IFT values were recorded until a stable value was reached. When IFT values fell below 1 $\text{mN m}^{-1}$ and stable droplet creation was not possible, values were set to 0 $\text{mN m}^{-1}$.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

B.H. and K.J. contributed equally to this work. The authors acknowledge Ms. Cornelia Weber for the preparation of actin filaments and Prof. Michael Grunze for helpful feedback and discussions. The authors acknowledge funding from the European Research Council, Grant Agreement no. 294852, SynAd, and the MaxSynBio Consortium, which is jointly funded by the Federal Ministry of Education and Research of Germany and the Max Planck Society. The authors also acknowledge the support from Klaus Tschira Stiftung, the German Science Foundation SFB 1129 and the Volkswagen Stiftung (priority call “Life?”). J.P.S. is the Weston Visiting Professor at the Weizmann Institute of Science and part of the excellence cluster CellNetworks at the University of Heidelberg. K.G. received funding from the European Union Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No. 792270, from the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany’s Excellence Strategy via the Excellence Cluster 3D Matter Made to Order (EXC-2082/1 - 390761711), and the Max Planck Society. K.J. thanks the Carl Zeiss Foundation for the financial support. The Max Planck Society is appreciated for its general support. Open access funding enabled and organized by Projekt DEAL.

Conflict of Interest

The authors declare no conflict of interest.
Keywords
actin, droplet-based microfluidics, Marangoni effect, motility, surfactants, synthetic biology

Received: August 12, 2020
Revised: September 22, 2020
Published online: November 16, 2020

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