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# All-Aqueous Assemblies via Interfacial Complexation: Toward Artificial Cell and Microniche Development

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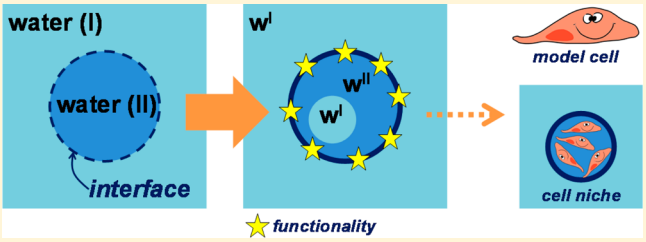
**ABSTRACT:** In nature, the environment surrounding biomolecules and living cells can dictate their structure, function, and properties. Confinement is a key means to define and regulate such environments. For example, the confinement of appropriate constituents in compartments facilitates the assembly, dynamics, and function of biochemical machineries as well as subcellular organelles. Membraneless organelles, in particular, are thought to form via thermodynamic cues defined within the interior space of cells. On larger length scales, the confinement of living cells dictates cellular function for both mammalian and bacterial cells. One promising class of artificial structures that can recapitulate these multiscale confinement effects is based on aqueous two-phase systems (ATPSs). This feature article highlights recent developments in the production and stabilization of ATPS-droplet-based systems, with a focus on interfacial complexation. These systems enable structure formation, modulation, and triggered (dis)assembly, thereby allowing structures to be tailored to fit the desired function and designed for particular confinement studies. Open issues for both synthetic cells and niche studies are identified.






## 1. INTRODUCTION

**1.1. Compartmentalization in Nature.** Throughout biology, the temporal and spatial coordination of components on various scales is needed to perform complex functions.[1−3] This coordination is achieved, in part, by compartmentalization to confine components or entities, control the local environment, and regulate material fluxes and reactions. Fundamental studies that address the formation, structure, and roles of compartments in nature have inspired materials scientists to create complex structures to recapitulate their important features and functions. If these bioinspired compartmentalized structures are used to manipulate proteins or other delicate cargo, then they must be formed from materials that minimize deleterious effects. Such a requirement has spurred interest in aqueous two-phase systems (ATPSs), which provide a rich platform for structure formation and are gentle hosts of bioactive components. ATPSs are composed of hydrophilic materials (polymers) that demix into two water-rich phases when their concentrations exceed critical values. Thermodynamically, this phase separation occurs when the entropic driving force that favors mixing becomes less than the enthalpic penalty that opposes it.[4] There are a number of pairs of polymers and proteins/polysaccharides that display this phenomenon; one commonly used pair is that of poly(ethylene glycol) (PEG) and dextran.[5] Historically, ATPSs have been explored as biological two-phase extraction media[6,7] and exploited as diagnostic tools.[8] ATPSs and traditional oil−water emulsions are similar in that the two have distinct compositions and the interface can be characterized by a finite interfacial tension. However, the interfacial tension between the two phases in an ATPS is significantly lower than that between oil and water because both phases are largely made of water. Because of such a low interfacial tension, the width of the interface is fairly broad (i.e., 5−10 nm). Furthermore, the inherent biocompatibility of ATPSs makes them particularly attractive for use as hosts to compartmentalize various biological and/or nonbiological components.

**1.2. Molecular Confinement: Self-Assembly within Cells.** Cells are the smallest form of life, and subcellular compartmentalization is central to life itself. The interior space of cells is divided into organelles or compartments in which specific tasks are executed and coordinated, ranging from DNA sequestration to energy production and lipid and protein formation. To perform these tasks, molecules must partition, react, and redistribute selectively from one organelle to another. Organelles can be membrane-bound; that is, they are encased in lipid bilayer membranes and bound to the cell membrane itself. Membrane-bound organelles are stable entities that are resistant to environmental fluctuations, can isolate cytotoxic reactions, and retain ions as a result of the presence of the lipid membranes.[9] These stably confined entities have inspired a wide and well-established body of work on lipid or polymer vesicles,[10,11] which has been shown to support broad ranges of cell-mimetic processes such as DNA transcription,[12] encapsulation,[13] and RNA/enzyme catalysis.[14]

There are other organelles that are not encased within a lipid bilayer. These membraneless organelles are fluid droplets composed of biomolecular condensates that are thought to form by phase-separation processes within the cell cytoplasm and are found in both prokaryotic and eukaryotic cells.

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Membraneless organelles are dynamic entities, sensitive to fluctuations in pH, temperature, and the local concentration of other biomolecules. Fundamental studies have begun uncovering mechanistic insights into their formation and function within the cell.[3,9,15−18] Membraneless organelle formation is understood in terms of biopolymer or protein solution thermodynamics; above a critical concentration, the system phase separates into protein-rich and protein-poor phases when enthalpic penalties overcome the entropic driving force for mixing,[2,9,15] resulting in a protein-rich aqueous compartment that is not bound by a membrane, surrounded by an aqueous medium. This compartment can selectively partition molecules in the protein-rich phase and exchange them with the cytoplasm in the cell interior and vice versa, such as RNA accumulation within the nucleolus and preribosomal particles exported from the nucleoplasm to the cytoplasm.[19] Related solution thermodynamic arguments apply to the phase separation of polyelectrolyte coacervate phases in aqueous solutions, which are also able to host proteins.[20,21] In such systems, the formation of coacervates between two interacting species (e.g., oppositely charged polyelectrolytes) results in liquid−liquid phase separation akin to that of ATPS. Because the interactions between the two species depend on various environmental conditions such as temperature, pH, and salt concentration, assembly and disassembly of coacervates can be triggered by such stimuli. Furthermore, in this context, stable ATPS droplets are prime candidates as vehicles for protocells or bioinspired cell mimics.[22] Such systems could serve as models for the membraneless organelle formation process and could aid in the elucidation of responses to cues such as curvature, compartmentalization, pH, temperature, and ionic strength.

The Keating group has made seminal contributions in this arena. They exploit the ATPS system of PEG and dextran encapsulated within giant unilamellar lipid bilayer vesicles (GUVs) to serve as model cytoplasm within a synthetic cell. Both PEG and dextran serve as model biopolymers that mimic the crowded cytoplasm.[23,24] Furthermore, under appropriate conditions, domains rich in dextran or PEG form and serve as model membraneless organelles within this model cytoplasm (Figure 1).[24−27] These synthetic cells, or protocells, are powerful tools for modeling cellular functions. Additionally, the formation of dextran-rich phases can be modulated on the basis of the initial composition of ATPS and solution conditions. This modulation is important in a protocell model system in which induced crowding can be turned on or turned off on the basis of external stimuli. Proteins partitioning from one phase to another can be tuned in response to external stimuli such as pH and temperature (Figure 2).[14,28,29] Such protein partitioning studies provide insights into related biological processes such as the partitioning and reaction of RNA in membraneless organelles. This research firmly establishes APTSs as appropriate model systems for studying and mimicking membraneless organelles.

In addition to these studies that exploit GUVs filled with ATPS, there has been significant activity focused on other ATPS droplets or capsules with differing levels of complexity, with increasing control over the size, shape, and composition of the final structure as well as the transport properties of the shell that encapsulates ATPS-based structures.[30−33] Moreover, the protocell concept has inspired materials scientists to imbue these assemblies with functionalities that go beyond biology by the incorporation of synthetic materials such as nanoparticles and other nonbiological materials. This feature article highlights

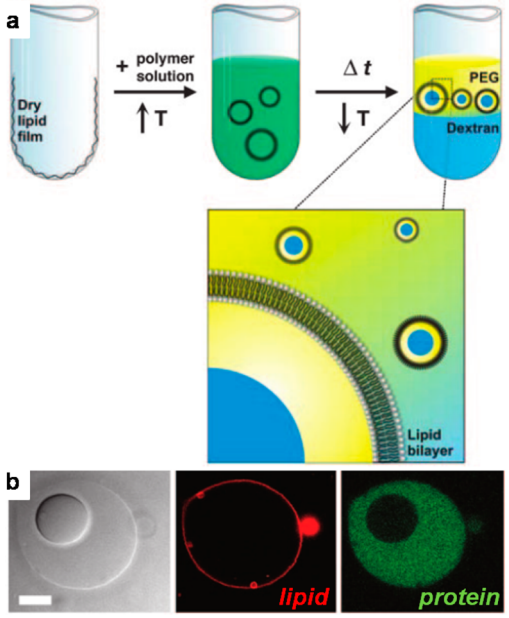


**Figure 1.** Lipid-stabilized PEG/dextran ATPS emulsion. (a) Emulsion-formation scheme. (b) Transmitted light (left) and fluorescent images of an emulsion droplet, prepared with a rhodamine-tagged lipid (center) and fluorescein-tagged streptavidin (right). Scale bar = 10 $\mu$m. Reprinted with permission from ref 25.

recent advances in the development of ATPS-based compartments and their potential applications. There have been some successes in creating ATPS Pickering emulsions[34−40] and the aforementioned studies in which ATPS droplets are formed within GUVs. For those who are interested in these topics, excellent reviews can be found in refs 26 and 51. Here, we focus on artificial membrane-like materials that formed via interfacial complexation between pairs of oppositely charged polyelectrolytes and pairs of nanoparticle and polyelectrolytes.

## 2. ALL-AQUEOUS ASSEMBLIES VIA INTERFACIAL COMPLEXATION

**2.1. Generation of ATPS Droplets and Dispersions.** To exploit ATPS droplets, reliable means to form them with control over their size and morphology are required, a task that presents unique challenges due to the low interfacial tension of aqueous−aqueous interfaces ($10^{-3}$−$10^{0}$ mN/m). The aqueous nature of both phases results in broad interfacial widths and concomitant low interfacial tensions.[41−43] Unfortunately, widely used microfluidic methods that rely on jet break up for drop formation of immiscible fluid mixtures such as oil and water systems[44] are not well suited to this purpose. The physics of drop formation by jet breakup relies on a balance of the viscous/inertial forces that form the jet and the interfacial tension that drives its breakup into droplets.[45−47] For very low tensions, the viscous and inertial forces dominate, and the jet remains intact.

To overcome this challenge, a number of techniques have been developed to introduce new effects to create water-in-water droplets based on ATPS as summarized in Figure 3 and Table 1.[5] A simple flow-focusing device that relies on gravity to drive the dispersed phase through the device at low flow rates and generate weak viscous and inertial forces enables water-in-water drop formation.[48] Despite the low droplet generation rate, the device creates monodisperse droplets with a coefficient


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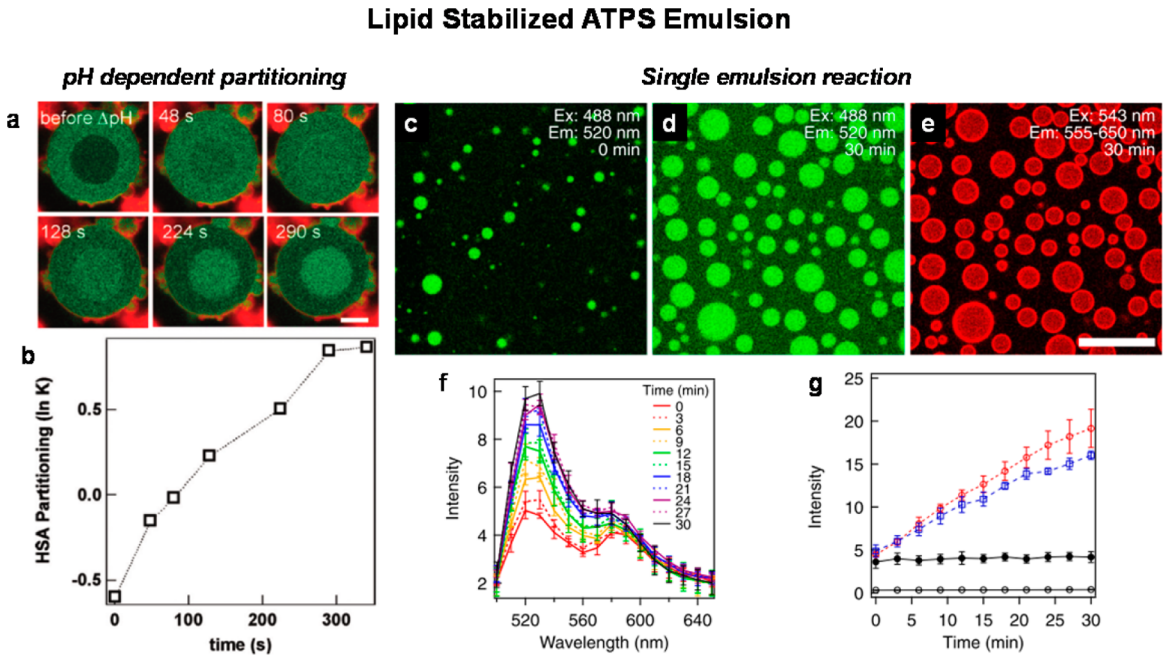


**Figure 2.** Protein partitioning and RNA cleavage reaction within liposome-stabilized ATPS. (a) Fluorescent human serum albumin (HSA, green channel) is localized in the PEG-rich phase at pH 6.5 and partitions to the dextran-rich phase upon an increase to pH 12. Red fluorescence is due to the rhodamine-tagged lipid. The scale bar is 5 $\mu$m. (b) Measured partitioning coefficient (ln $K$) with respect to time after pH change: negative ln $K$ indicates PEG-phase affinity and positive ln $K$ indicates dextran-phase affinity. (a, b) Reprinted with permission from ref 29. (c) Initial fluorescein channel image and (d) 30 min after reaction has started, demonstrating an increase in donor intensity in the PEG-rich phase. (e) The rhodamine channel at $t$ = 30 min includes acceptor fluorescent and liposome tags. The scale bar is 25 $\mu$m and applies to (c–e). (f) Fluorescence emission spectra (ex: 488 nm) from the PEG-rich phase, which come both from a decrease in quenching (due to the reaction) and an increase in the PEG-phase concentration due to repartitioning. (g) Reaction progress monitored in the PEG-rich phase by summing the intensity from 520 and 530 nm to capture the donor peak, without lipid membranes (red data), with lipid membranes (blue data), and without enzyme degredation (no reaction, black line). (c–g) Reprinted with permission from ref 28.

of variation (CV, i.e., polydispersity) of 1–10% (Figure 3a). Higher droplet-generation rates in microfluidic devices are enabled by perturbing the inlet stream of the dispersed phase periodically to induce droplet breakup with the Rayleigh–Plateau instability (Figure 3b,c). In this scheme, the stream can be pulsed (Figure 3b)[49] or disturbed mechanically[50,51] or piezoelectrically[52,53] (Figure 3c) to create monodisperse droplets.

There is a particularly ingenious method that bypasses the challenges associated with ultralow interfacial tension to create water-in-water emulsions from ATPSs. In the all-aqueous electrospray (AAE) technique,[5,54] the drop-forming aqueous phase is sprayed into the air, taking advantage of the elevated surface tension of the air–water interface to form dispersed droplets; once formed, the droplets fall through the air into a bath containing the external aqueous phase (Figure 3d). AAE relies on charging the dispersed phase and placing an electrode of opposite charge away from a capillary; a jet forms that rapidly disintegrates in air into discrete droplets smaller than those formed in a simple gravitational dripping regime. Like other microfluidic techniques, the droplet size and the rate are highly controllable based on a number of variables such as the field strength, capillary diameter, and injection rate.

**2.2. Stabilization and Functionalization of ATPS Droplets.** Once generated, to prove useful as biological hosts for substantial times, the all-aqueous drops must be stabilized. Once again, the ultralow tension of the ATPSs poses unique challenges because most stabilization strategies developed for oil–water systems rely on the appreciable interfacial tension between the two phases and exploit the distinct chemical differences of the dispersed and continuous phases.[55] These include the adsorption of surfactants, macromolecules, or stabilizing ligands and the trapping of dense layers of particles at the interface to impart repulsion and suppress coalescence. Unlike oil–water-based mixtures, ATPS lacks the significant discontinuity in polarity across the interface, which substantially reduces the driving force for the adsorption of amphiphilic molecules. Moreover, the energy of attachment is directly proportional to the interfacial tension; therefore, low interfacial tension leads to low driving force for attachment. Therefore, to stabilize all-water emulsions, more careful material selection is typically required because of the lack of a strong driving force to induce the adsorption of molecules and materials to the interface. Here, we focus on the interfacial complexation of oppositely charged species to stabilize these ATPS-based emulsions.

In this approach, oppositely charged polyelectrolytes (PEs) are introduced into the two aqueous phases. Ideally, the PE in the drop phase meets and complexes with the PE from the external phase at the interface. In all-aqueous systems, PEs are soluble in either phase, affording tremendous flexibility in material selection but also presenting challenges. Because the PEs can freely enter either the drop or external phases, equilibrium partitioning plays an important role.[33] Furthermore, the mass flux of the PEs must be balanced so that they meet and complex at the interface to create a stable membrane (Figure 4b). If the fluxes are unbalanced, then complexation occurs away from the interface, either within the dispersed phase (Figure 4c) or in the continuous phase (Figure 4a) as demonstrated using two strong PEs, poly(diallyldimethyl-


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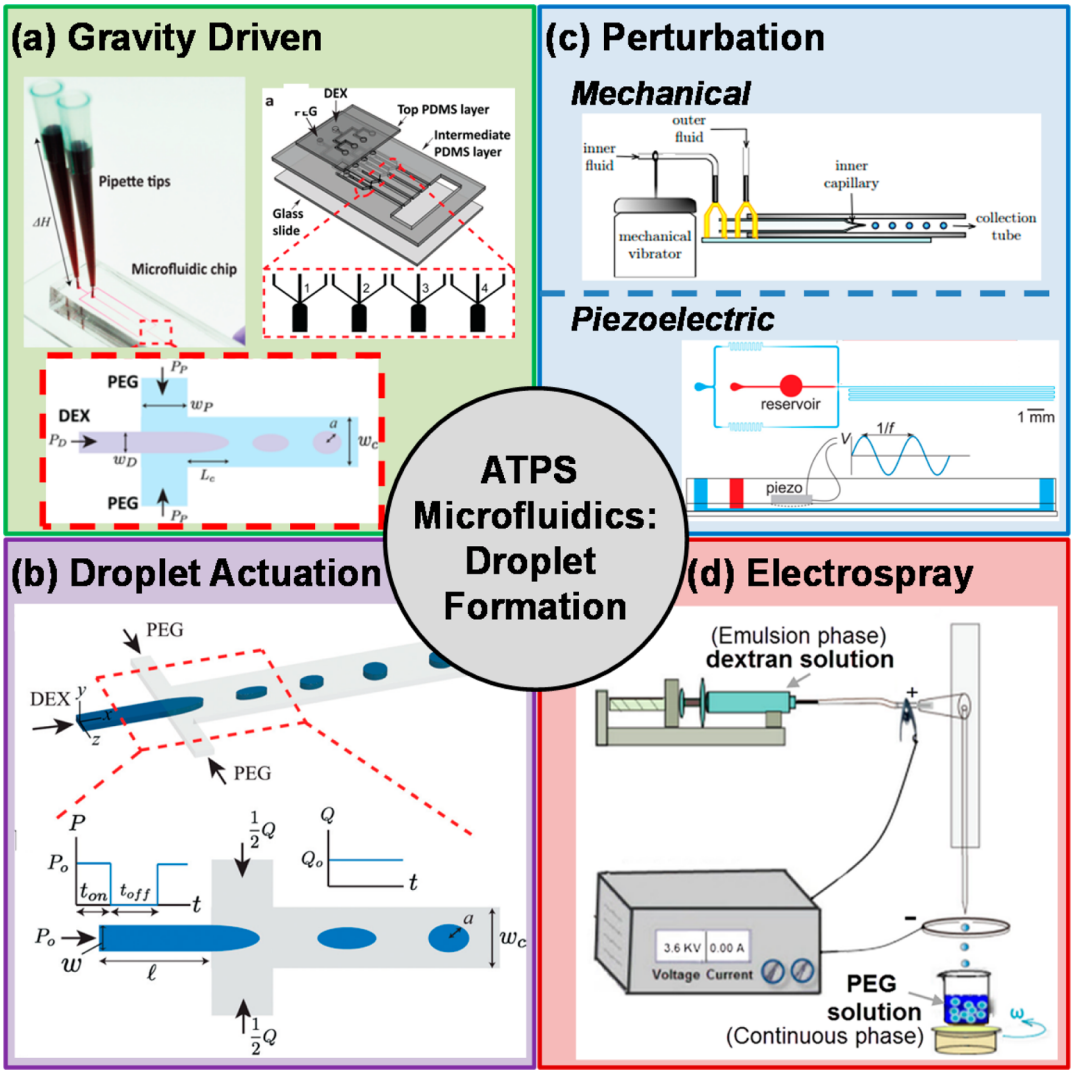


**Figure 3.** Summary of ATPS microfluidic techniques used to create monodisperse water-in-water droplets. (a) PDMS device in which the inlet pressure is controlled by the hydrostatic pressure of fluids in inlet reservoirs. Low inlet pressure enables droplet breakup due to lower viscous/inertial forces. Reprinted with permission from ref 48. (b) The inner phase (DEX) is pulsed periodically to induce droplet breakup. Reprinted with permission from ref 51. (c) (Mechanical) The tubing of the inner fluid is perturbed with a mechanical vibrator to induce droplet breakup within a glass capillary device. Reprinted with permission from ref 50. (Piezoelectric) A piezoelectric bending disc is positioned at the inlet channel into the PDMS device. Sinusoidal voltage is applied, to which the bending disc periodically contracts and relaxes, resulting in a constricted and relaxed channel width, leading to droplet breakup. Reprinted with permission from ref 53. (d) The emulsion phase is charged and injected into a glass capillary device, which is then pulled out into the continuous phase through an oppositely charged electrode. Reprinted with permission from ref 54.

**Table 1. Summary of All-Aqueous Microfluidic Techniques**

| method | generation rate (drops/s) | droplet diameter ($\mu$m) | limitations |
|---|---|---|---|
| (a) gravity | 1 orifice: 1−154 orifices: ∼50 | 10−40 | low production rate |
| (b) droplet actuation | ∼170 | ∼50 | requires an actuator with a microfluidic device |
| (c) perturbation | ∼400 | ∼70 | requires an external vibrational source |
| (d) electrospray | up to 1000 | 50−2500 | electric field may cause material degradation |

ammonium chloride) (PDADMAC) and poly(styrenesulfonate) (PSS). The relative fluxes of the two species and therefore the location where complexes form can be controlled by changing the ratio of the two PEs present in the ATPS, as summarized in Figure 4d. Interfacial complexation under balanced fluxes of the two PEs results in stable microcapsules (PSS/PDADMAC = 0.5:1 and 1:1 in Figure 4d), whereas complexation within the droplet leads to the formation of microgel or coacervate particles made of the two PEs (rightmost image in Figure 4d); the composition of these particles can be influenced by the equilibrium partitioning in each phase, as illustrated in Figure 5.[30,33] Polyelectrolyte flux is influenced by the molecular weight, charge density, concentration, and viscosity of the host phase. PSS, which favorably partitions to the dextran phase but was originally placed in the continuous PEG phase (Figure 5a−c), results in microcapsule formation, and fluorescently tagged PE is found only at the PEG−dextran interface. In contrast (Figure 5d−f), microgel particles will form if the PSS is originally placed in the droplet phase with all else constant, and fluorescently tagged PE is


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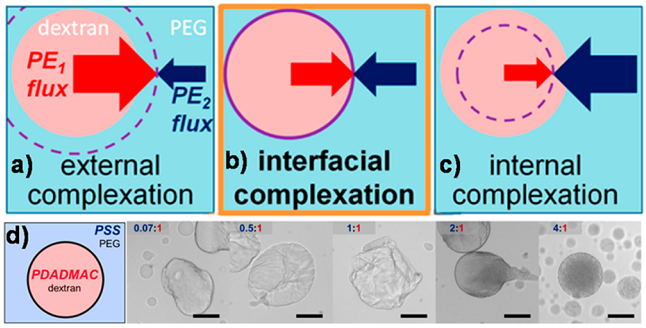


**Figure 4.** Flux-dependent interfacial polyelectrolyte complexation in water-in-water droplets generated by AAE. (a–c) Schematics of flux-dependent complexation locations: (a) external phase, (b) interface, and (c) internal phase. Fluxes are tuned on the basis of relative concentrations between the two polyelectrolytes. (d) Representative optical images of complexation between poly(diallyldimethylammonium chloride) (PDADMAC) from the dextran droplet phase and poly(styrenesulfonate) (PSS) from the continuous PEG phase. The ratios represent the relative molar charge ratios of PSS/PDADMAC based on 0.5 wt % PDADMAC. Left to right images undergo external, then interfacial (wrinkled), and then internal complexation. Scale bars are 100 $\mu$m. Reprinted with permission from ref 30.

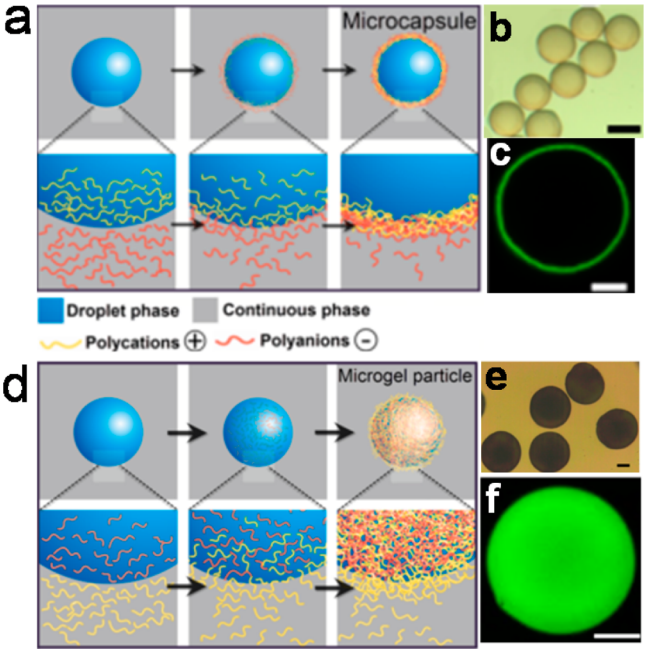


**Figure 5.** Microcapsule and microgel formation from strong and weak polyelectrolytes in water-in-water droplets generated by AAE. (a) Schematic of microcapsule formation from interfacial complexation. (b) Optical image of poly(styrenesulfonate) (PSS) and poly(allylamine hydrochloride) (PAH) microcapsule. Scale bars are 300 $\mu$m. (c) Confocal image of the PSS-PAH capsule, with the fluorescent signal from FITC-PAH. The scale bar is 50 $\mu$m. (d) Schematic of microgel particle formation based on internal complexation. (e) Optical micrograph of PAH-PSS microgel particles. (f) Confocal image of a PAH-PSS particle fabricated with FITC-PAH. Scale bars are 100 $\mu$m. Reprinted with permission from ref 33.

found throughout the droplet phase. The complexation of two oppositely charged PEs has also been induced in the middle phase of water-in-water-in-water double emulsions prepared using a microfluidic device. In this scheme, one polyelectrolyte is added to the outer phase, and an oppositely charged PE is added to the inner phase; the middle aqueous phase serves as a complexation zone (Figure 6[56]). Although this drop-formation mechanism is more complex, it affords control of the complexation location without relying as heavily on balancing the fluxes of the two PEs.

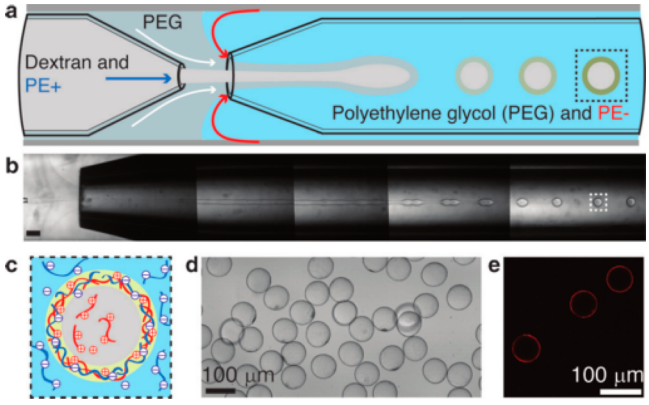


**Figure 6.** Polyelectrolyte complexation in the middle aqueous phase of a water-in-water-in-water double emulsion. (a) Schematic of the glass capillary microfluidic device used to fabricate microcapsules. (b) Optical micrograph of droplet breakup. (c) Illustration of the complexation of PDADMAC and PSS occurring in the middle water phase, which is the complexation zone. (d) Optical micrograph of final capsules. (e) Fluorescent image of capsule shells labeled with the ethidium homodimer. Scale bars are 100 $\mu$m. Reprinted with permission from ref 56.

These stabilization techniques based on interfacial complexation have several advantages, including rapid complexation, diverse materials options, and scalable single-step processing. The PE/PE microcapsules have flexible and elastic membranes that can support pressure differences, as evidenced by their ability to wrinkle and expand under osmotic stresses. The polyelectrolyte complex layer is also permeable, allowing chemical exchange between the interior and exterior of the microcapsule. Finally, these capsules are stimuli-responsive; the electrostatic interactions can be modulated using external stimuli such as pH and ionic strength.

ATPS-derived capsules can be imbued with enhanced functionality via the introduction of nanoparticles (NPs) to provide catalytic, luminescent, magnetic, or other useful properties. The incorporation of NPs in the shell can be accomplished by inducing interfacial complexation between a PE of one charge and an oppositely charged NP, similar to the process used for PE/PE interfacial complexation. A demonstration of this strategy using $SiO_2$ nanoparticles and PDADMAC in ATPS has been reported.[31] The inclusion of $SiO_2$ NPs results in brittle outer shells rather than flexible membranes as evidenced by their rupture under osmotic stress, and the mechanical properties of the NP-laden shell can be modulated, as discussed below.

The incorporation of NPs not only changes shell mechanics and affords new functionality but also provides an unexpected pathway to the scalable formation of encapsulated all-aqueous double emulsions. When a PE is added to the droplet (dextran) phase and $SiO_2$ NPs are added to the continuous (PEG) phase, the formation of a PE/NP microcapsule was accompanied by the spontaneous inclusion of a droplet made of the outer aqueous phase (PEG) within the PE/NP microcapsule. The


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formation of such encapsulated double emulsions results from the rapid formation of a rigid PE/NP complex layer (Figure 7a), followed by the displacement of water from the inner

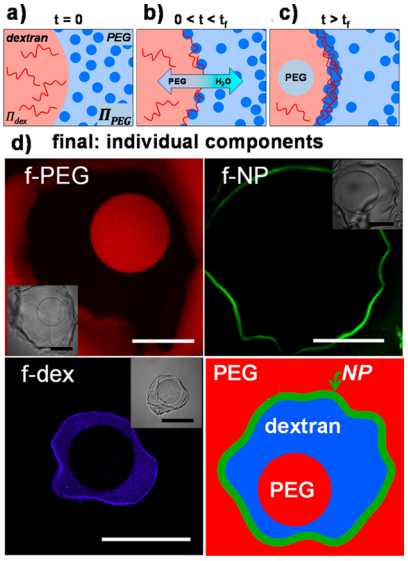


**Figure 7.** Interfacial complexation of polyelectrolyte and nanoparticle results in the spontaneous formation of an encapsulated double emulsion, named AWE-somes. (a–c) Illustration demonstrating the inner drop inclusion mechanism. (d) False-colored confocal images and compiled schematic of individual components. (Red, f-PEG) Rhodamine-tagged 20 kDa PEG. (Green, f-NP) Tetramethyl-rhodamine-core silica NP. (Blue, f-dex) Rhodamine-tagged 70 kDa dextran. All scale bars are 100 $\mu$m. Reprinted with permission from ref 31.

phase and the concomitant influx of the continuous phase through the highly permeable PE/NP shell due to an osmotic pressure imbalance (Figure 7b) until the permeability of the PE/NP shell is significantly reduced (Figure 7c). By using fluorescently labeled components, the inner drop was confirmed to be a membraneless PEG drop, and the outer shell was confirmed to be composed of the PE and NPs, as shown in Figure 7d. These all-water emulsion bodies, or AWE-somes, have several features that resemble the basic structure and functionality of the cell. The spontaneously formed droplets within the AWE-some do not have membranes, much like membraneless organelles found in the cell. In some ways, these structures resemble internal membraneless droplets formed within lipid vesicles by other means, including phase separation within vesicles and phase separation events followed by budding.[57−59] Notably, molecules can be selectively compartmentalized in the included droplet and undergo reaction in response to an agent that permeates the PE/NP shell, as described in Section 2.3.

The structure and properties of AWE-somes can be further enhanced and tailored for applications requiring mechanical and chemical robustness and integrity. AWE-somes prepared via the electrospray method are from tens of micrometers to millimeters in diameter with irregular nonspherical shapes that can be attributed to the mismatch in the osmotic pressures of the two phases and the deformation and relaxation of the electrosprayed water droplets as they plunge into a bath of the external phase during interfacial complexation.[32] As noted above, the AWE-somes formed via the interfacial complexation of oppositely charged NP and PE are surrounded by rigid shells that are prone to brittle rupture upon mechanical or osmotic perturbation. The possibility of modulating the shell properties while maintaining the spontaneous formation of AWE-somes was explored by inducing interfacial complexation between a mixture of negatively charged $SiO_2$ NP and PSS in the external (PEG) phase with an oppositely charged PE, PDADMAC, in the droplet (dextran) phase.[32] This scheme leads to the formation of what we call compound AWE-somes that incorporate all three charged species. These compound AWE-somes enable rapid, single-step structure formation as PE/NP AWE-somes while enabling PE/PE microcapsule shell-like toughness, making them resistant to rupture against mechanical and osmotic stresses. As shown in Figure 8, the inclusion of

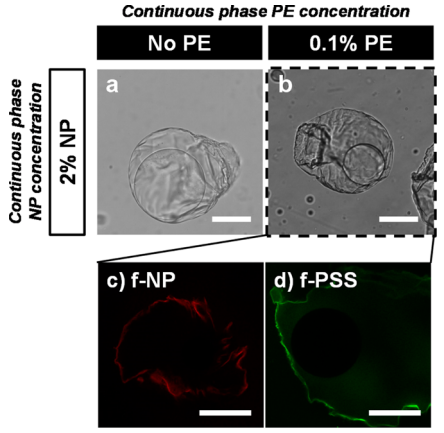


**Figure 8.** Microcapsule with tunable structure and properties by interfacial complexation between a mixture of polyanion and anionic NP in the continuous phase and a cationic polymer in the dispersed phase. (a) AWE-some made by spraying 15% dextran and 0.5% PDADMAC into 10% PEG and 2% $SiO_2$ NP. (b) By including both anionic PE and $SiO_2$ NP in the continuous phase, capsules form with both the NP (c) and the PE (d) in their shells, imbuing stimulus resistance and enhanced flexibility while maintaining the AWE-some double-emulsion structure. Scale bars are 100 $\mu$m. Reprinted with permission from ref 32.

even a small amount of PE in the continuous phase results in the incorporation of both anionic species in the membrane. Moreover, these compound AWE-somes are able to maintain their structure in high-ionic-strength solutions, unlike PE/NP AWE-somes that disintegrate in such an environment. These features are important, especially for the application of AWE-somes as protocells in which the shell may be exposed to varying temperature, pH, and ionic strength.

**2.3. Stabilized ATPS Droplets as Cell Mimics.** Artificial cells or protocells are materials designed to recapitulate key features of biological cells, including the selective partitioning of components, supporting reactions in subcellular compartments, and selective and controlled permeability through the encapsulating membrane.[22,60] Some examples of such cellular functions include RNA synthesis, amplification of DNA, and enzymatic cascading product formation. In addition to the lipid-stabilized ATPSs,[27,29,61] the AWE-somes highlighted above have a significant potential to achieve many of these features. Within AWE-somes, small molecules can diffuse and partition within the different compartments, as evidenced by the selective partitioning of small fluorescent molecules rhodamine and fluorescein (Figure 9a). Furthermore, a simple quenching experiment has shown that these structures are excellent


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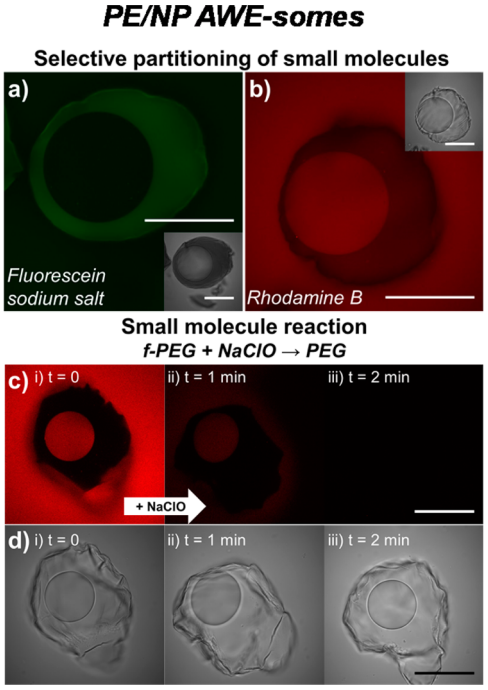


**Figure 9.** Diffusion, partitioning, and reaction of small molecules within PE/NP AWE-somes. Fluorescent images showing the preferential partitioning of (a) fluorescein salt to the dextran phase and (b) rhodamine B to the PEG phases. (c) Confocal microscopy images of the fluorescence quenching of f-PEG (red signal). NaClO, added to the continuous PEG phase, diffuses into the AWE-some lumen, and reacts with the f-PEG, quenching the fluorescent signal. (d) No loss of capsule integrity is observed in bright-field images. Scale bars are 100 $\mu$m. Reprinted with permission from ref 31.

candidates for inducing reactions while enabling phase selectivity. Fluorescently labeled PEG was included in the internal droplet by slow exchange with the external phase. Subsequently, a fluorescence quencher, NaClO, was added to the continuous phase. The quencher readily diffused through the outer shell of the PE/NP AWE-some and quenched the fluorescence within the internal droplet as shown in Figure 9. These simple demonstrations show some of the basic processes of selective partitioning and reaction within subcellular organelles that occur within the cell and are recapitulated within these structures. The membrane is permeable to macromolecules of moderate size; therefore, the sequential addition of reactive materials is possible, which would be essential in simulating the dynamic nature of biological cells.

Significant potential to further enhance AWE-some functionality could be achieved via a layer-by-layer approach, as has been demonstrated for PE/PE microcapsules, which were imbued with additional PE layers,[33] and for NP/PE shells, which were decorated with lysozyme, a known antibacterial agent. Cellular processes such as the recognition and internalization of molecules and materials indeed depend on the membrane-associated proteins and macromolecules. The incorporation of functional molecules that can communicate across the membrane is a step in the direction of imparting such functions to AWE-some cell mimics.

**2.4. Cellular-Scale Confinement: Cellular (Micro)-niches.** Whereas Section 2.3. addresses how AWE-some presents the basic structure and functionality that are inspired by subcellular compartments and organelles, the confinement of biological cells plays important roles on other larger length scales as well. The confinement and environment of growing cells play important roles in the cell fate. One example includes stem cells that respond to environmental cues that affect their differentiation pathway and function;[62] the stem cell environment is known as the cell niche. Environmental cues also impact bacterial cells; microbial microniches within biofilms influence community dynamics and survival.[63] Artificial systems that mimic cell niche and bacterial microniche studies must

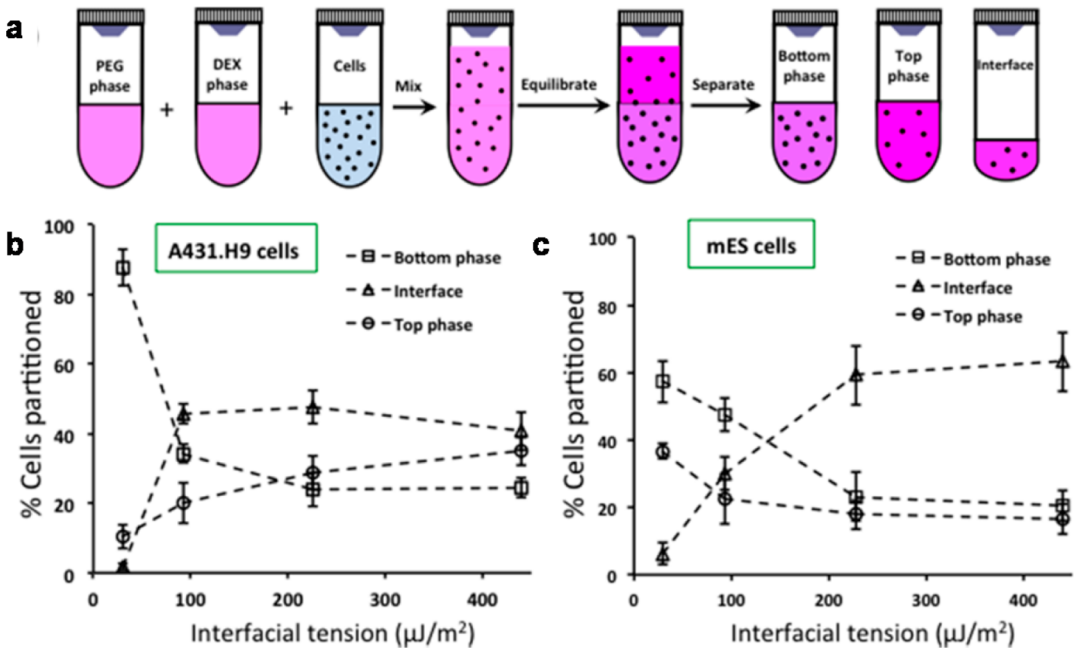


**Figure 10.** Cell partitioning as a function of interfacial tension in the PEG/dex ATPS. (a) Schematic of experimental setup. (b, c) Cell partitioning behavior for two different cell types as a function of interfacial tension. Reprinted with permission from ref 71.



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allow for the control and manipulation of the cell environment and the means to probe the cell response.

Although there are successful examples of microbial microniches within oil−water systems,[64,65] ATPS-derived structures such as those discussed above can be versatile vehicles as cell niches or microbial microniches. Such systems can sequester cells, allow the partitioning and exchange of biomolecules between multiple compartments, and allow for a controlled environment. Indeed, driven in part by the need to pattern and localize cell growth for applications in niche studies and tissue engineering,[66−70] there is now a significant understanding of how cells and cell materials partition within ATPSs (Figure 10).[71,72] During the equilibration of an ATPS made of PEG and dextran, mammalian cells partition to either the PEG-rich or dextran-rich phase or the interface (Figure 10a). The differential partitioning of cells is a function of interfacial tension, controlled by ATPS composition, and cell type, which affects the cell surface chemistry (Figure 10b,c). Similarly, studies have demonstrated that ATPS can be used to pattern cells into (micro)niches to enable the investigation of cell interactions.[73,74]

The structures formed via interfacial complexation in ATPS described in this article may be particularly interesting as niches or microniches for cell encapsulation because they allow for the rapid exchange of media or solvent while protecting their cargo from external forces. PE/PE microcapsules have been shown to successfully encapsulate bacteria and maintain moderate growth with minimal media. Moreover, the cellular function, in this case the cell viability, could be probed by adding a fluorescent probe to the external phase and letting it permeate into the PE/PE capsule lumen as shown in Figure 11. The growth of cells can be improved upon by including nonbacteriocidal membrane-forming components such as polypeptides.

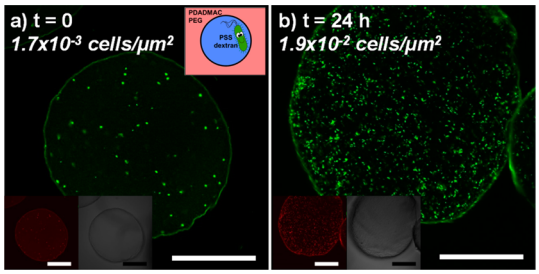


**Figure 11.** Encapsulation of *Pseudomonas aeruginosa* in a PE/PE microcapsule fabricated with dextran-PSS electrosprayed into PEG-PDADMAC and resuspended in minimal media. (a) Initially, the cell density is $10^{-3}$ cells/$\mu$m$^2$. Incubated at 37 °C for 24 h. (b) The cell density has increased by an order of magnitude. The green signal corresponds to live and dead cells stained with Syto 9. The red signal corresponds to dead cells stained with propidium iodide. All scale bars are 100 $\mu$m. Reprinted with permission from ref 43.

Mammalian cells have been successfully encapsulated in unstable ATPS droplets;[48] therefore, stable ATPS structures should be able to support mammalian cells and also enable the long-term study of cell dynamics. In this case, compound AWE-somes are extremely promising candidates because of their stability against changes in the salt and pH conditions. Additionally, the possibility of imparting new functionality through the incorporation of synthetic nanoparticles and functional proteins provides an exciting and yet-to-be explored potential to control the cellular fate within the cellular niche.

## 3. CONCLUSIONS AND OUTLOOK

ATPSs provide numerous attributes, in particular, biocompatibility, making them a versatile platform for producing compartments that can form the basis for cell mimicry and (micro)niche studies. This feature article highlighted the recent advances in understanding and exploiting interfacial complexation in ATPS to enable the production of microcapsules and all-water emulsion bodies (AWE-somes). The thermodynamics as well as transport and interfacial phenomena in ATPS must be taken into consideration to enable control over the resulting structures in this approach. The results summarized here clearly demonstrate that there is a significant potential for using these ATPS-derived structures for more complex biomimicry and biological studies.

Moreover, new opportunities to create systems with functionality and properties that go beyond those of the living cell exist. The inclusion of weak PEs whose degree of ionization depends strongly on the local pH, for example, will enable AWE-somes with pH-sensitive structure and properties. The incorporation of canonical and noncanonical biomacromolecules such as proteins, DNA, or polypeptides will imbue ATPS-derived AWE-somes with additional biological functionality and responsiveness. Also, the burgeoning field of intrinsically disordered proteins and their phase behaviors presents new opportunities to build membraneless organelles in AWE-somes.[2,15,75] Another potential variation on the capsules highlighted in this article is to include hydrogel-like droplet phases, analogous to the alginate complex systems.[76−81] Such high-viscosity microenvironments are important for stem cell niche applications in which the mechanobiology of cells plays a major role in determining the cell fate.[82] Synthetic nanoparticles can also imbue these AWE-somes with useful functionality. Once biomolecules such as proteins and enzymes are incorporated into AWE-somes, these capsules can serve as an unloading vessel in which a stimulus can induce the release of the complexed materials. To enable such applications, it will be imperative to study the impact of interfacial complexation on the activity of enzymes or proteins.[56,83,84] Another intriguing possibility that has been barely explored is the potential of inducing interfacial complexation into aqueous mixtures that are out of equilibrium or that are triggered to undergo phase separation. The quenching of a phase-separating system via interfacial complexation could potentially lead to bicontinuous structures akin to those recently developed on the basis of bicontinuous interfacially jammed emulsion gels (bijels).[85] In these cases, the phase separation requires a spinodal-like decomposition and nearly instantaneous interfacial attachment and jamming.

To fully realize these potentials, more detailed studies of various fundamental aspects involving interfacial complexation in ATPS are necessary. The growth mechanism of these interfacial complex layers is yet to be understood. Results thus far indicate that these interfacial complex layers can grow to be quite thick (>100s of nm), suggesting that there is continued complexation of the two oppositely charged species beyond the first few molecular layers. Such a process likely accompanies the diffusion of PEs within the growing layer. Whether this process is thermodynamically or kinetically limited remains an open question. Also, a more detailed understanding of the effect of assembly conditions and materials on the mechanical and transport properties is currently lacking and warrants future investigation. One of the key features that various biological


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membranes exhibit is their fluidity. In fact, fluidity is an essential property that enables various cellular functions such as focal adhesion formation and the regulation of membrane permeability.[86,87] Thus, efforts to understand and engineer the fluidity of the interfacial complex membranes in ATPS would be extremely valuable. The field of layer-by-layer (LbL) assembly has accumulated a tremendous amount of knowledge and understanding that is likely to be highly relevant.[88] However, it will also be important to discern the differences between LbL-based complexes and those prepared via interfacial complexation. All of these efforts would also substantially benefit from recent advances in understanding the charge-based interactions and complexation based on computational and modeling-based efforts.

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### Notes

The authors declare no competing financial interest.

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