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Design and Application of Stimulus-Responsive Droplets and Bubbles Stabilized by Phospholipid Monolayers

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Abstract

Biomimetic colloidal particles are promising agents for biosensing, but current technologies fall far short of Nature's capabilities for sensing, assessing, and responding to stimuli. Phospholipid-containing cell membranes are capable of binding and responding to an enormous variety of biomolecules by virtue of membrane organization and the presence of receptor proteins. By tuning the composition and functionalization of simulated membranes, soft colloids such as droplets and bubbles can be designed to respond to various stimuli. Moreover, because lipid monolayers can surround almost any hydrophobic phase, the interior of the colloid can be selected to provide a sensitive readout, for example in the form of optical microscopy or acoustic detection. In this work, we review some advances made by our group and others in the formulation of lipid-coated particles with different internal phases such as fluorocarbons, hydrocarbons, or liquid crystals. In some cases, binding or displacement of stabilizing lipids gives rise to conformational changes or disruptions in local membrane geometry, which can be amplified by the interior phase. In other cases, multivalent analytes can promote aggregation or even membrane fusion, which can be utilized for optical or acoustic readout. By highlighting a few recent examples, we hope to show that lipid monolayers represent an extremely versatile biosensing platform that can react to and detect biomolecules by leveraging the unique capabilities of phospholipid membranes.

Graphical Abstract

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1. Introduction

While many strides have been made in the development of biosensors and other responsive structures, there is still a considerable gap between current synthetic technologies and the potential for smarter components offered by Nature. Living systems can detect small numbers of analytes in a noisy background, perform logic functions on incoming data, and transduce signals to other parts of an organism. In turn, these complex functions are performed by clusters and networks of cells that regulate the flow of energy, electrons, and biochemicals inside and outside their boundary. In order to begin to access the rich biomimetic functional space offered by cell networks, we must first seek to leverage some of the functionality of each individual membrane, which provide many important functions to cells, including long-term stability, replication, compartmentalization of chemical processes, the ability to interact with other cell and membranes, and selective uptake of nutrients.

While cell membranes are composed of a complex mixture of phospholipids, sphingolipids, cholesterol, and transmembrane proteins, most basic synthetic lipid membrane mimics have at least two components: a majority fraction of phospholipid and a minority fraction of polymer amphiphile. The phospholipid imparts basic stability to the membrane by stacking their tails into tight interactions stabilized by the hydrophobic effect. For instance, one of the most common dominant components of synthetic lipid membranes, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), is a saturated double-tailed phospholipid with 16 carbon atoms on each tail. Because DPPC's melting temperature (T_m) $\sim 41^\circ\text{C}$, at room temperature the lipids form a gel phase in which the tails fit together and form a hydrophobic barrier of ~ 1.5 nm per layer [1]. With each increase in $-\text{CH}_2$ group in the acyl chain of a phospholipid, the van der Waals interaction between the chains increases. For example, Garg *et al.* calculated that, assuming 2D hexagonal packing of acyl chains in a monolayer, the cohesive energy (~ 500 mJ/m²) of the acyl chains in a DPPC (C16:0) monolayer increases by almost 50% for DBPC (C22:0) [2]. Because alkyl tail length also increases membrane rigidity, unsaturated lipids and sterols may be added to produce synthetic vesicles that more closely mimic cell membranes in their rigidity and permeability.

In living cells, mixtures of lipids with different tail structures produce so-called “rafts,” which are responsible for organizing various transmembrane proteins for vital functions. Similarly, a mixture of synthetic lipids may also undergo lateral lipid phase separation, in which multiple phases, including gel (solid) and expanded (liquid), coexist on the colloidal interface. As was shown elegantly by Veatch and Keller, a mixture of saturated phospholipids (DPPC), unsaturated phospholipids (DOPC, POPC), and cholesterol formed unilamellar vesicles with a varied phase diagram [3]. Variation of the relative compositions of these components revealed the formation of nanoassemblies of ordered or disordered phase lipid and cholesterol in the vesicle bilayer; sterol-rich regions, for instance, often segregate into a L_o (liquid-ordered) phase. This lateral phase separation mimics lipid rafts containing heterogeneous collection of preferentially associated domains of lipids, proteins, and sterols [4]. The local geometry and rigidity of these rafts is thought to determine the types of proteins accommodated therein. While majority of phase behavior studies have been conducted on bilayer vesicles, the trends observed have been found to hold for monolayers

as well below the transition temperature of the predominant phospholipid in the membrane (usually, DPPC) [5].

Finally, synthetic monolayers on nanoparticle surfaces must contain at least a minority fraction of a polymer amphiphile. Because hydrophobic colloids are subject to aggregation, coarsening, and/or ripening over time, a steric barrier is required to prevent or hinder two colloids from overcoming the potential energy barrier to irreversible adhesion. While a polymer brush layer is preferred to prevent bridging between two colloids, the main requirement is that the polymer remains attached to the nanocolloidal interface over relevant time scales. In practice, most synthetic lipid membranes are stabilized by a conjugate of a polymer, usually poly(ethylene glycol) (PEG) conjugated to a saturated phospholipid (DPPE or DSPE). The added advantages of PEG are their resistance to fouling by serum proteins, compatibility in many solvents (including water), and the ability to add functionality on the free terminus for conjugation of other biomolecules, including targeting ligands or nucleic acids.

In the following sections, we will summarize new developments in leveraging synthetic lipid monolayers to impart functionality to hydrophobic soft colloids (Figure 1). The reason for focusing on hydrophobic colloids, as opposed to liposomes or vesicles, is that the hydrophobic synthetic cores may be utilized to create mechanisms for “turning on” the signal immediately upon binding, as will be described below. Initially, we will discuss methods by which phase changes that occur in the phospholipid monolayer can be transduced into an observable signal. Such changes include lipid reorganization and membrane bending. The second part of this review will highlight new outcomes in association of soft colloidal particles. The underlying themes of this review will be the ways in which subtle interactions with and changes in the phospholipid monolayer can be converted into a detectable signal. For example, we will focus on the phenomena of local shape change in the form of reorganization, disruption, poration, lysis, aggregation, and fusion of lipid membranes, all of which has been shown to occur under different conditions via interactions with physiologically relevant proteins [6–9].

2. The Heterogeneous Phospholipid Membrane: Interactions with a Single Monolayer.

Cells are able to transduce information across their membranes through the reorganization of lipids, sterols, and proteins in the membrane [4], all while maintaining membrane integrity and separation of interior and exterior cellular components. In Nature, signal transduction is rarely a monovalent interaction; rather, many components in the mobile lipid structure are able to reorganize their structures to bind to or sense a signaling molecule or analyte. One of the major challenges in utilizing these interactions for synthetic biosensors is the ability to transform the binding interaction into a signal that can be detected in a background of unreacted colloids. Because of the requirement for a “turn-on” signal, here we will focus specifically on the utilization of liquid crystal droplets (polarized optical microscopy) and bubbles/droplets (ultrasound imaging).

2.1. Sensing Lipid Reorganization and Desorption Through Changes in Liquid Crystal Alignment

There have been considerable strides in the development of thermotropic liquid crystal (LC) emulsions as robust reporters for sensing changes in lipid packing or shape and amplifying these effects into optically detectable signals, resulting in detection limits as low as pg/mL [10–13]. The inherent property of LC birefringence allows for different optical appearances of LC orientation under cross-polarized light. Usually, at the interface between a low molecular weight LC and an aqueous phase, the native LC orientation is planar; i.e. the LC director is aligned parallel to the interface. Upon adsorption of certain types of surfactants, the orientation of a LC can transition to homeotropic; i.e., the LC director is aligned perpendicular to the interface [14–16]. Such observations pertaining to the change of LC orientation based on interfacial composition at the surface have led to applications in protein detection via macromolecular adsorption [17] or DNA hybridization [18,19].

LC emulsions are potentially useful as sensors because of their high surface area, mobility, dispersibility, compatibility with microfluidics, and even as an agent embedded within hydrogels [20–23]. Rather than parallel or homeotropic, the curved surface causes the LC to either align in the “radial” direction, or normal to the interface, or without radial symmetry (e.g., with axial symmetry) in a “bipolar” configuration (see below). The radial-to-bipolar transition and vice-versa has been increasingly employed of late as a platform for sensing of various biomolecules predominantly by block copolymer coated LC droplets. While outside the specific scope of this work, it is worthwhile to mention that polymer-stabilized droplets have been successful in detecting molecules like urea [24], cholesterol [25], glucose [26], IgG [27], among others. Phospholipid monolayers, however, have presented an opportunity for increasing LC emulsion stability [10,13]. However, phospholipids are not simply innocent bystanders in these biosensing technologies, as surfactant tail length also appears to significantly affect LC orientation: a short-chain surfactant like OTAB (eight carbon atoms in the chain: C8) preserves a planar LC alignment at a surface, whereas the longer DTAB (C12) and CTAB (C16) produce a homeotropic alignment [14]. Low-melting point phospholipids like DLPC, DPPC, and DOPC (C18:1) also induce a homeotropic LC orientation [10,11]. Thus, displacement of lipids by certain surfactants has promoted LC transition in either direction. For example, Bera and Fang tested droplets of the commonly used liquid crystal 5CB, stabilized by a shell of the fluorescently labeled phospholipid NBD-PE, in the presence of bile acids: in this case, lithocholic acid (LCA) disrupted the fluorescent dye (NBD) conjugated PE lipid molecules, and ultimately desorbed sufficient quantities to change the droplet appearance from radial to bipolar, providing a system for LCA detection down to 10–70 μ M (Figure 2A) [11]. Similarly, Abbott and coworkers showed that interface-active mimics of bacterial Lipid A showed strong adsorption to LC emulsions [28].

An exciting direction for phospholipid-stabilized LC emulsions is the ability to impart biochemical specificity through interactions between the analyte and lipid. For example, earlier work on surfaces showed a transition of LC orientation from homeotropic to planar in 1–2 h upon binding of specific proteins like phospholipase [15,29] and neutravidin [29]. However, non-specific adsorption of proteins like albumin, cytochrome-c, and lysozyme, at a

1 μM concentration, apparently does not alter the 5CB arrangement [15]. Similarly, Gupta *et al.* formulated DLPC-coated LC emulsions, which detected the enzyme phospholipase A₂ (PLA₂) down to 2 nM (Figure 2B) [30]. The cleaved lipid tails desorbed from the monolayer, thus lowering the surface anchoring energy of the LC mesogens and causing a radial to bipolar transition [30]. In another example, cancer cells were detected when adhesion of folate ligands caused an LC transition [31]. Potentially, this concept could be plausibly extended to any kind of protein with the ability to interact with LC. For example, small peptides with specific secondary structure could be detected on LC emulsions due to the individual effects on LC orientation [32]. Finally, Abbott and co-workers also recently demonstrated the application of enzyme (thermolysin) sensitive LC droplets, coated with peptide-polymer amphiphiles, wherein the droplets turned from a bipolar to radial configuration on processing of the shell material by the enzyme (Figure 2C) [33]. Customization of these peptide-lipid conjugates presents the most promising approach for lipid-stabilized LC emulsions because of its ability to be customized to many different interactions.

2.2. The Phospholipid Monolayers on Fluorocarbon Bubbles and Emulsions.

Highly distinct lateral lipid phase separation has been observed at the fluorocarbon-water interface due to the immiscibility between the fluorocarbon phase and alkylated lipid tails. Fluorocarbon nanodroplets (NDs) for ultrasound imaging contain a volatile, liquid perfluorocarbon core and sizes in the hundreds of nanometers [34,35]. These “phase-shift” emulsions can be vaporized to form microbubbles by the application of heat or high intensity focused ultrasound, or even diagnostic ultrasound for low-boiling condensed perfluorocarbon internal phases [36]. Such NDs could first be allowed to extravasate through leaky tumor vasculature into the interstitial space, and then vaporized to generate microbubbles for contrast imaging or therapy; this strategy has been explored in recent years in the fields of intratumoral imaging, drug and gene delivery, ablation therapy, and embolotherapy, among others [37].

Recent work by Chattaraj *et al.* explored the lateral phase separation and domain formation in mixed component shells (in the vein of unilamellar vesicles, containing saturated, unsaturated lipids and cholesterol) on perfluorocarbon droplets and the consequent effects on ease of droplet vaporization [38]. Originally, Borden *et al.* had shown that phospholipid lateral phase separation was enhanced on perfluorocarbon microbubble monolayers (Figure 3A) [39]. This trend appears to hold for perfluorocarbon liquid droplets as well. For instance, a 1:1 composition of DPPC:DOPC (saturated:unsaturated) lipids (with 5% cholesterol) in the droplet shell showed the presence of multiple small solid domains surrounded by a liquid phase (Figure 3B). A DPPC monolayer, on the other hand, forms a largely ordered phase. This study observed that for the largely ordered single-lipid monolayer required more acoustic energy for vaporization than the relatively more heterogeneous mixed lipid monolayer. This is thought to be because of nucleation sites in the form of tiny solid domains being created by the lateral phase separation in the monolayer. This work illustrates how future designs may exploit this phenomenon for designing particles where interactions with stimuli can alter the shell phase separation and cause a detectable acoustic response.

2.3. Chemical Modifications of Lipid Structure to Enact Structural Changes to the Monolayer

Another approach to biosensing is to utilize changes in chemical structure of the phospholipid shell to effect changes in mechanical properties. Some recent examples in this area have leveraged the mechanical stiffness of microbubbles (MBs) as ultrasound contrast agents capable of biosensing. In the context of biomedical applications, MBs are 1–10 μm gas bubbles encapsulated by a stabilizing layer of lipids, polymers, or proteins [40], where the gas is commonly an inert perfluorocarbon. These MBs, being excellent scatterers of sound waves in the range of 1–18 MHz, have been extensively explored for applications in contrast imaging of various organs and blood vessels, targeting to tumors or other tissues for specific imaging and drug delivery, sonoporation, blood-brain barrier disruption, and delivery of gases like oxygen and NO [40–42]. The mechanism of acoustic contrast by MBs comes not merely from their elastic scattering but also the nonlinear size oscillations that occur when the MBs are stimulated at their resonance frequency [40]. The resonance frequency in turn ranges from 1–18 MHz depending on a number of properties, including MB size, gas heat capacity ratio, and shell stiffness; however, if the bubble shell is stiff enough, the incident acoustic pressure will be insufficient to drive bubble oscillation, and the nonlinear response (and therefore acoustic contrast) will be dampened or even removed entirely [43].

Recently, the shell structure has been utilized to enact changes in bubble shell stiffness. Nakatsuka *et al.* pioneered this technique by first attaching complementary aptamers onto DSPEPAA molecules incorporated into a DPPC shell of the bubble: the strands would crosslink among themselves, elevating the rigidity of the bubble shell (Figure 4A) [44]. As opposed to free bubbles, these crosslinked bubbles produced limited response under mechanical indices in the range of 0.2. On addition of a decrosslinking strand to the solution, the bubble shell regained its flexibility, and produced a higher acoustic response. This technique was used to detect thrombin molecules in non-occlusive blood clots (Figure 4B): thrombin was able to de-crosslink complementary anti-thrombin aptamers crosslinked on the MB shell, and was detected down to about 20 nM in solution [45,46].

More recently, Lux *et al.* used a different mechanism for detecting the same biomarker [47]. Activatable cell-penetrating peptides (ACPP) were used instead of aptamers to detect thrombin in the diagnosis of deep vein thrombosis. The cysteine in ACPP binds easily to commercially available maleimide conjugated lipids, making the functionalization of MBs much easier than the conjugation of DSPE and PAA followed by EDC-NHS reaction chemistry to attach aptamers as done previously. ACPP-MBs were then challenged with concentrations of thrombin in the 10–100 nM orders of magnitude, which cleaved the polyanionic inhibitory domain while revealing the polycationic cell penetrating peptide. This allowed activated MBs to adhere and accumulate around the thrombus, eliciting a 4.7 fold increase in ultrasound signal than non-activated and control MBs.

3. Soft Colloidal Aggregation and Fusion: Inter-Membrane Interactions

Nanocolloidal aggregation has been at the forefront of biosensing for a number of years. For example, gold nanoparticles possess different optoelectronic properties in different

functionalized and aggregated states, which has given rise to numerous sensors based on colorimetric aggregation assays [48]. Similar strategies have been utilized for detecting DNA, proteins, small molecules and metal ions by aggregation of gold and silver nanoparticles [49]. In this part of the review, we first summarize recent advances in biomimetic approaches towards droplet aggregation, then discuss ways to leverage these associations for analyte detection.

3.1. Biomimetic Mechanisms of Intermembrane Adhesion.

While functionalizing lipid membranes on particles with ligands/receptors can cause inter-membrane association, the distribution of such entities are often heterogeneous. Lateral phase separation studies have shown the formation of domains rich in particular types of lipid/protein molecules based on overall composition. Cells are able to communicate through the establishment of intracellular junctions and signaling pathways. An elegant mimic of this process was recently shown by Pontani *et al*, who designed biomimetic emulsions to simulate adhesion of cells mediated by E-cadherin (Figure 5A) [50]. Cadherins are transmembrane, calcium-dependent proteins that promote cell-cell adhesion for cell signaling and motility. The amount of ambient calcium affects the cytoskeletal behavior: while *cis* interactions occur within a cytoskeleton under low calcium levels, higher levels facilitate the *trans* interactions necessary for intercellular adhesion. However, the complexity of the cytoskeletal structure led the authors to ask whether the *cis* clustering of cadherin molecules could enhance the *trans* interaction between two cells. Initially, experiments were done without calcium: even then, weak *trans* adhesion was observed. Since the mimicking droplet monolayer was designed to have a similar stiffness as the outer leaflet of a cell membrane, the high surface tension therein is likely to facilitate inter-cell cadherin-cadherin parallel bridges. These bridges, in fact, arise at cadherin-rich zones due to passive recruitment of cadherin molecules by the membrane into specific domains, as dictated by the membrane composition: for instance, incorporation of cholesterol causes lateral accumulation of cadherin-carrying lipids due to separation of liquid ordered and disordered phases (Figure 5A). Such results highlight the potential in designing biomimetic structures, where ligand or receptor clustering on the lipid shell could improve sensitivity to the system to the stimulus.

Additional work has shown that similar lipid rearrangements occur with other adhesion mimics such as biotin-streptavidin or DNA [51,55]. A previous study by Pontani *et al* showed that two lipid droplets with Texas Red labeled streptavidin attached to the surface form bright fluorescent surface patches at adhesion sites (Figure 5A) [51]. This localization of the streptavidin on adherent droplets was irreversible when the driving force of adhesion was withdrawn. This demonstrates that reorganization of the lipid membrane and aggregation between two lipid-coated particles may not be mutually exclusive events, and that membrane geometry can be leveraged to enhance association, which can lead to further shape changes and response. The flexibility of chemical structures that can promote adhesion is expected to lead to more complex aggregates with high shape control, potentially for logic-mediated responses. For example, Zhang *et al* recently showed the directed self-assembly of droplets, in which prehybridized DNA-coated micron-sized lipid-silicone oil droplets were introduced to ‘initiator droplets’ that displaced one of the DNA strands on the

former, leading to sequential and controllable association of the droplets (Figure 5B) [52]. As a result, oligoaggregates were obtained rather than simple dimers or large clusters. Another study by van der Wel *et al* developed droplets with a polymerizable TPM oil phase, which then served as a platform for studying lipid monolayer coverage [56]. Simple addition of DNA linkers conjugated on lipids was able to functionalize the TPM droplets and were mobile on the droplet surface. Interactions with complementary polystyrene particles formed controllable droplet-particle aggregates. The particles, unlike the linkers, were immobile on the surface, thus making the droplet a mimic for a solid support, and a way for formulating shape-tuned permanent clusters.

3.2. Biomimetic Membrane Fusion.

In some cases, aggregation of lipid layers can lead to fusion of multiple nanocolloids into single, larger colloids. In this case, local hydrophobic ‘pockets’ are opened between two stressed lipid membranes, lowering their line tensions at the domain phase boundaries [57,58]. In cells, fusion is driven by the activity of fusogenic proteins called SNAREs (Soluble N-ethylmaleimide-sensitive factor Attachment protein Receptors). Cells utilize the SNARE complex for dictating how organelles find a specific part of the membrane. The process which is responsible for bringing two lipid monolayers together by overcoming the entropic loss is theorized to be the formation of a parallel four-helix bundle consisting of different SNARE proteins [59]; this ‘zippering’ process has recently been shown to be dynamic, with the cooperativity and number of SNARE proteins involved varying based on biophysical properties (curvature) of membrane mimics [60].

As of this writing, lipid fusion has not been leveraged as often as aggregation. However, a recent study by Mohan *et al.* exploited this process to detect an analyte via content mixing between two or more cell-mimicking emulsions (Figure 5C) [53]. This work was inspired by earlier advances made by Hook and coworkers in the design of liposomal mimics containing cholesterol-DNA conjugates to trigger directional fusion [61]. Mohan *et al.* prepared fusogenic lipid-coated liquid crystal droplets by incorporating specifically designed oligonucleotides in the droplet monolayer, such that they would hybridize through a ‘head-to-head’ coupling. As mentioned before, during the process of association of two membranes, membranes first undergo adhesion to form aggregates, and if the adhesive forces are strong enough, a pore will form between the two layers and the vesicles will fuse, leading to content mixing. This ‘pulling together’ of the two monolayers enabled the mixing of the internal LC phases containing two different dyes for two sets of droplets; the colocalization analysis of the two dyes through confocal microscopy provided a platform for biomolecular sensing. In this particular case, thrombin-sensitive DNA sequences detected the molecule in solution down to 10–100 nM. The SNARE-inspired complementary DNA sequences caused a 40% fusion as opposed to 3–5% non-specific fusion for non-functionalized droplets. Further studies are needed to investigate the lipid monolayer mechanical properties as well for better tunability of the fusion process. While docking, hemi-fusion, and fusion events have been extensively studied for lipid vesicles, relatively little is known about similar events for droplets. Ghimire *et al* posited that the entire process of lipid-coated droplet fusion is rate-limited by the docking step which ensues from water-shedding between two droplets. Having a POPC monolayer on triolein droplets reduced

interfacial tension slowing down the water-shedding process and hence the entire droplet association event, in spite of the fact that the subsequent physical fusion was relatively quick. In about 3% of fusion cases, lipid-coated droplets exhibited a short-lived hemifusion step, which was not observed in bare triolein droplets. It follows that, in addition to affecting the rate of monolayer breakage and fusion, tuning the lipid composition can also be made to dictate flow across the membrane [62]. For example, a recent report by Lopez *et al.* found that permeability of water across droplet interface bilayers increased considerably with two or more double bonds in the acyl chain, while decreasing almost linearly with increasing saturated acyl chain length from C14 to C22, a result that could be exploited to control the extent of content mixing [63].

3.3. Obtaining a Detectable Signal from Intermembrane Adhesion.

While considerable effort focused on utilizing the proximity of nanostructures for optical coupling, the shape and structural changes in the lipid layer are another potential way to create stimulus-responsive materials for acoustic sensing. A few years back, Shpak *et al.* showed that non-linear acoustic waves focus at a point inside a droplet due to i) the curvature of the droplet causing wave refraction, and ii) the mismatch in acoustic impedance between the droplet core and the surrounding medium [64]. This superharmonic focusing was shown to be responsible for nucleation events occurring inside the droplet. This finding was leveraged by Chattaraj *et al.*, who showed that droplet aggregates were more susceptible to vaporization than single droplets (Figure 5D) [54]. To test this hypothesis, nanodroplets were functionalized with biotin and incubated in a solution of varying amounts of streptavidin. While droplets are generally responsive to HIFU as a stimulus, the threshold energy required to generate this signal varies. It was shown in this study that, at a low incident intensity at which free droplets do not vaporize, aggregation caused droplets to vaporize and produce signal, peak negative pressure being constant. Through this technique, streptavidin, as a model biomarker, was specifically detected down to picomolar levels within minutes (Figure 5D). The concept of aggregation-induced reduction in vaporization threshold was reinforced by a study by Guo *et al.* wherein sub-micron droplets were aggregated in a microfluidic chamber with a standing acoustic wave, followed by testing the vaporization threshold of aggregated vs non-aggregated droplets [54]. As expected, aggregated droplets produced signal at a lower incident peak negative pressure at a fixed intensity. The property of aggregation leading to improved acoustic response has been found for other structures as well, including bacteria-derived gas vesicles [65].

4. Conclusion and future prospects.

This review aims to show how the combination of phospholipid monolayers and hydrophobic soft colloids can combine to create new technologies for biosensing. In particular, we have focused on the ability of lipid membranes to undergo complex changes in phase behavior, biomolecular adhesion, and organization in response to analyte challenge. First, the complex lateral lipid phase behavior in monolayers has been shown not only as a mimic for lipid rafts but also as a reorganization event that can be detected. Second, the association of monolayers on adjacent droplets could be used not only to mimic cellular

adhesion but also to change how nanodroplets with volatile cores could be vaporized under high intensity focused ultrasound.

Despite the significant advances in this area, there are two major roadblocks that must be overcome for lipid-coated soft colloids to gain broader acceptance for biosensing. First, because of the need to detect small amounts of analyte in a background containing many particles, the specificity of interaction between analyte and lipid must be optimized. Nonspecific interactions, especially in physiological media, are inevitable in biosensing, yet Nature has developed ways to ignore random, weak associations and interactions and instead react to specific binding events. The scientific path forward should combine fundamental studies to understand the mechanism of interactions of different proteins with cell membranes, followed by the design of increasingly tunable biomimetic particles. Another possibility would be to utilize existing cell membranes that have been placed on nanoparticles, a strategy that has been utilized successfully in drug delivery [66].

On the detection side, the future of the field rests in the creation of new ways to signal the interaction of synthetic lipid monolayers with biomolecular analytes. In this review, we have presented two major methods to detect positive associations in a noisy background: polarization optical microscopy of liquid crystal emulsions and acoustic detection of ultrasound contrast agents. Other approaches toward sensing nanoparticulate associations have utilized optical or fluorescence approaches, such as resonance energy transfer or surface enhanced Raman spectroscopy. We also suggest the possibility of utilizing content mixing to synthesis detection agents *in situ*. For example, Chattaraj *et al.* showed that lipid-coated vegetable oil droplets containing either a reduced (deactivated) fluorescent dye or an oxidizing agent produced fluorescent clusters upon association with vascular endothelial growth factor, serving as a platform for its detection[67]. This possibility could result in the synthesis of other types of optical, magnetic, or electrical reporters that could provide high signal-to-noise biosensing compatible with in-solution, on-chip, or even *in vivo* applications.

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Abbreviations.

5CB	4-Cyano-4'-pentylbiphenyl
CTAB	Hexadecyltrimethylammonium bromide
DPPC	1,2-dipalmitoyl-sn-glycero-3-phosphocholine
DLPC	1,2-dilauroyl-sn-glycero-3-phosphocholine
DOPC	1,2-dioleoyl-sn-glycero-3-phosphocholine
DPPE	1,2-dipalmitoylsn-glycero-3-phosphoethanolamine

DSPE	1,2-distearoyl-sn-glycero-3-phosphoethanolamine
DSPE-PEG2000	1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000]
DTAB	Dodecyltrimethylammonium bromide
LCA	Lithocholic Acid
NBD-PE	1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (ammonium salt)
OTAB	Octyltrimethylammonium bromide
POPC	1-palmitoyl-2-oleoylglycero-3-phosphocholine
TPM	3-(trimethoxysilyl)propyl methacrylate

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(* denotes Important Paper; ** denotes Very Important Paper)

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Highlights.

- One of the challenges for in solution biosensing is utilizing interactions at the exterior of a colloid to induce a detectable signal in a noisy background.
- Lipid monolayers on droplets and bubbles can impart biomimetic function to synthetic colloids.
- Liquid crystals can amplify interactions with lipids for optical detection.
- Ultrasound represents an important and underused modality for detection with high signal-to-noise.
- Future challenges will focus on utilizing more faithful cell mimics for complex responses to stimuli.

Figure 1.

Schematic of mechanisms of biosensing discussed in this review. The first part will discuss biomimetic interactions between analytes and the lipid shell, causing a change in lipid phase behavior that can be detected by interactions with the colloid itself. The second part will discuss how biomolecule-driven, biomimetic interactions between colloids can be utilized for detection of fluid-filled colloids.

Figure 2.

(A) Lithocholic acids (LCAs) can be detected via their displacement of lipids on liquid crystal emulsion surfaces. From Ref. [11] (B) Binding of enzymes such as phospholipase A2 to peptide lipids and resultant lipid degradation leads to realignment of liquid crystals within emulsions. From Ref. [30] (C) Polymer-peptide conjugates stabilizing liquid crystal droplets is a potential universal platform for molecular detection. From Ref. [33]

Figure 3.

(A) Fluorescence microscopy images of microbubbles stabilized with DSPE and DSPE—PEG2000, with DiI as a fluorescent labeling agent. From Ref. [39] (B) (Left to right) Fluorescence microscopy, transmission electron microscopy, and HIFU-mediated CPS-mode ultrasound imaging of perfluorohexane nanodroplets stabilized by either (top) DPPC and DSPEPEG2000 or 47.5:47.5:5 DPPC:DOPC:Chol and DSPE-PEG2000. From Ref. [38]

Figure 4.
(A) Schematic of aptamer displacement from microbubble surface to effect changes in shell stiffness, with resultant ultrasound detection ability. From [45]. (D) Imaging of microbubbles in (B) in a flow chamber with (top) and without (bottom) clotting of whole blood. From [45].

Figure 5.

(A) Left: Fluorescence imaging of emulsion droplets showing streptavidin localization at adhesion zones, From [51]. Right: Imaging of cadherin distribution on droplet surface, showing enhancement in adhesion zone. From [50] (B) Utilization of DNA as an adhesion agent allows for formation of controlled chain nucleation and growth. From [52] (C) Schematic of fusogenic DNA leading to droplet fusion by reversing one strand sequence to promote same direction hybridization From [53]. (D) Aggregation of volatile, biotinylated perfluorocarbon droplets with increasing streptavidin concentration allowed selective ultrasonic vaporization of droplet clusters as compared to single droplets as a mechanism for in situ biosensing. From [54].