Trends in Biotechnology

CellPress

Review

Designing Artificial Cells towards a New Generation of Biosensors

Margrethe A. Boyd [1] and Neha P. Kamat [1,2,3,4,*,@]

The combination of biological and synthetic materials has great potential to generate new types of biosensors. Toward this goal, recent advances in artificial cell development have demonstrated the capacity to detect a variety of analytes and environmental changes by encapsulating genetically encoded sensors within bilayer membranes, expanding the contexts within which biologically based sensing can operate. This chassis not only acts as a container for cell-free sensors, but can also play an active role in artificial cell sensing by serving as an additional gate mediating the transfer of environmental information. Here, we focus on recent progress toward stimuli-responsive artificial cells and discuss strategies for membrane functionalization in order to expand cell-free biosensing capabilities and applications.

Highlights

Advances in synthetic biology have facilitated the development of cell-based and cell-free biosensors, enabling detection of molecular signals ranging from chemical contaminants to disease markers.

Artificial cells have emerged as a platform to combine the sensing activities of cell-free sensors with certain membrane functions demonstrated in cell-based sensors, including molecular containment, protectivity, and small molecule gating.

Recently, artificial cells have been designed to sense environmental molecules and initiate genetically-encoded responses. These sensors often utilize protein expression of membrane pores to release signaling molecules in response to a received input, facilitating communication with live and artificial cells.

Progress in membrane engineering will allow the chassis to serve as an active participant in artificial cell sensing.

Biosensing as a Critical Tool to Maintain Human and Environmental Health

In environments ranging from natural ecosystems to living organisms, small molecule **analytes** (see Glossary) and nanoscale forces serve as important markers of disease, pollution, and contamination. Unfortunately, these signals can be challenging to detect and monitor due to technological tradeoffs in analytical sensitivity, specificity, or deployment. With the expansion of modern agriculture and manufacturing techniques, as well as global health crises due to pollution and disease, the development of **biosensors** that allow for improved speed and accuracy of molecular detection in a variety of settings is critical for our ability to maintain human and ecological health. Accordingly, improved biosensing technologies are needed in fields including public health, food safety, agriculture, forensics, environmental protection, and homeland security [1].

Many traditional biosensing techniques, including nucleic-acid-based [2], antibody-based [3], and electrochemically based [4] biosensing, are exquisitely sensitive, but can be prohibitively expensive to develop and operate, require significant training, and use equipment that often makes point-of-detection sensing difficult [5]. Inspired by these limitations, biologically based sensors have emerged as an alternative that uses **genetic circuits** derived from living organisms to detect environmental signals. This approach has allowed the rapid development of new sensing platforms, which have shown great promise towards cost-effective, portable sensing [6,7]. Recently, progress has been made in the development of stimuli-responsive **artificial cells** - structures which recapitulate these genetically-encoded sensing pathways within a biologically-inspired material **chassis** – which act as self-contained biological sensors. The design of artificial cells provides an opportunity to bridge functions brought forth by synthetic biology and biomaterials, an intersection which promises to bring about unprecedented advances in biosensing. Here, we discuss progress toward chassis functionalization for improved biosensing, particularly with regard to membrane engineering, and discuss recent advances toward the development of genetically-encoded, stimuli-responsive artificial cells.

[1]Department of Biomedical Engineering, Northwestern University, Evanston, IL, USA
[2]Center for Synthetic Biology, Northwestern University, Evanston, IL, USA
[3]Chemistry of Life Processes Institute, Northwestern University, Evanston, IL, USA
[4]www.nehakamat.com

*Correspondence: nkamat@northwestern.edu (N.P. Kamat).
[@]Twitter: @NehaPKamat

Biologically Based Sensing: Cell-Free, Whole-Cell, and Artificial-Cell Approaches

Approaches to develop biologically-based sensors use strategies from either **top-down synthetic biology** or **bottom-up synthetic biology**, which encompass the redirection of sensing behaviors in living cells (**whole-cell sensor**) or the extraction and isolation of biological

Key Figure

Whole-Cell and Cell-Free Approaches Converge to Form Artificial Cell Sensors

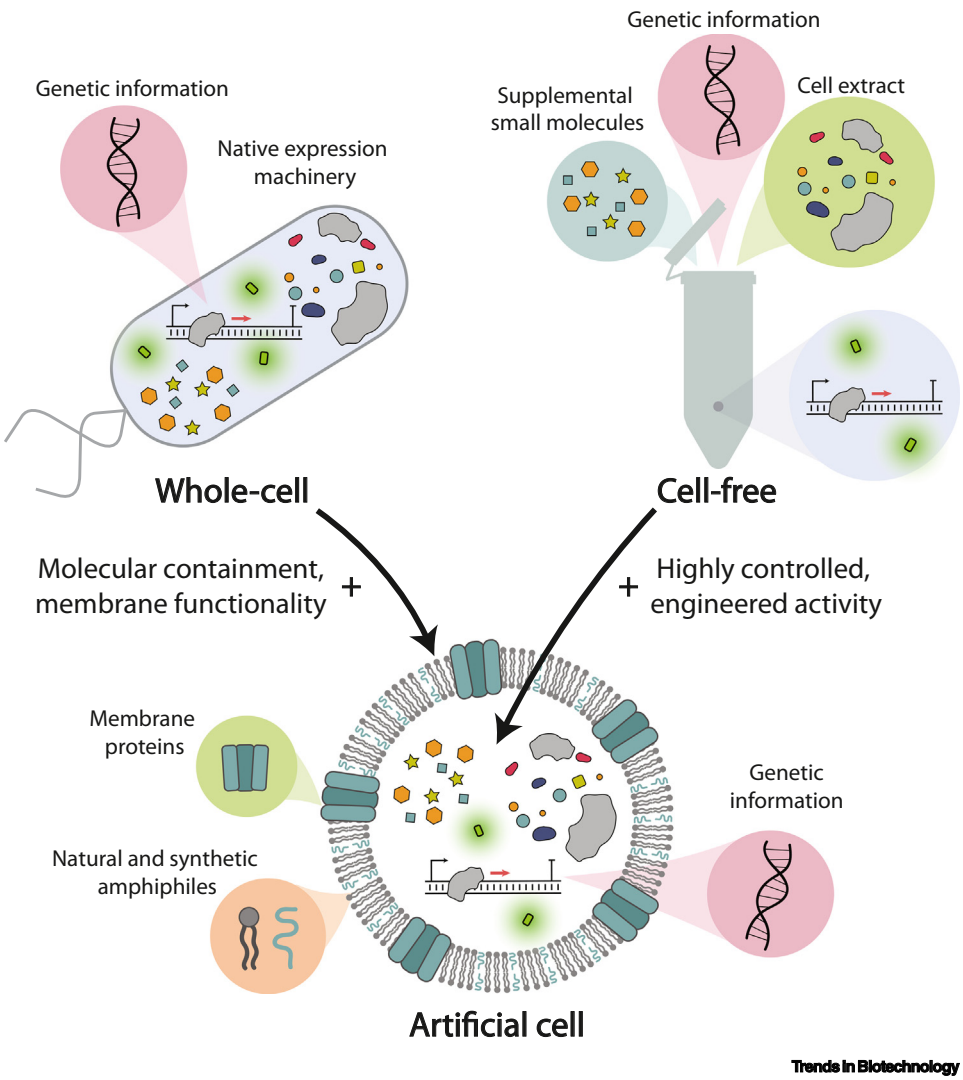

Figure 1. Artificial cells incorporate aspects of both whole-cell and cell-free biosensing strategies, including natural and synthetic membrane components and highly regulated, genetically encoded molecular sensors, in order to create a self-contained sensing environment. Through this combination, they recapitulate certain membrane functions of whole-cell sensors with the highly controlled genetic programs characteristic of cell-free biosensors. By including sensing-specific genes and limited reagents for protein synthesis, the risk of biocontainment may be significantly reduced and protective and gating features of the cell membrane can be re-introduced.

Glossary

Acylated homoserine lactones: membrane-permeable quorum-sensing molecules often used in artificial cell studies and bacterial communication pathways. The AHL N-3-(oxohexanoyl)-homoserine lactone (3OC6HSL) in particular has been explored in numerous artificial cell contexts.
Analyte: a substance, often a chemical or small molecule, that is detected, measured, or analyzed.
Artificial cell: an enclosed structure composed of compartmentalized bioactive molecules, which is capable of carrying out an essential activity of life – for example, sensing, signaling, communication, or growth/division.
Biosensor: an analytical device which uses biological components to detect the presence of a specific target molecule.
Bottom-up synthetic biology: a strategy that aims to assemble biomimetic systems from isolated components – synthetic or natural – in order to carry out biological activities.
Cell-free sensor: a bottom-up approach that uses transcription and translation machinery extracted from the cellular environment in order to carry out engineered, genetically encoded behaviors *in vitro* in response to a specific analyte.
Cell-free protein synthesis: the ability to express protein in the absence of living cells, resulting from the extraction of the cell's transcription and translation machinery into an *in vitro* environment.
Chassis: a structural component that houses the molecular components required for gene expression. In the case of artificial cells, this is often a bilayer membrane.
Giant unilamellar vesicle: micron-scale vesicles which are often used for artificial cell studies due to their cell-like size.
Genetic circuit: a genetically encoded assembly controlling the production of DNA, RNA, and proteins, which allows a system or cell to perform signal processing functions by turning a specific input into a desired output.
Lamellarity: the number of bilayers present in a vesicle membrane. A vesicle in which only one bilayer membrane is present is considered unilamellar.
Liposome: a vesicle composed of lipids.
Matrix effects: effects of sample or buffer components interfering with a signal, for example, by inhibiting transcription or translation in CFPS.

machinery from cells (**cell-free sensor**), respectively (Figure 1, Key Figure). Recent progress in whole-cell sensing has led to the development of cell-based sensors for insecticides [8], antibiotics [9], water contaminants [10], disease markers [11,12], heavy metals [13,14], and bacterial colonization *in vivo* [15]. Cellular transcription and translation machinery has also been extracted and harnessed to create **cell-free protein synthesis (CFPS)-based sensors** for viral infection [7,16,17], heavy metals and chemical contaminants [18–20], herbicides [21,22], date rape drugs [19], and clinically relevant biomarkers [23,24]. When it comes to deployment, however, these sensors have encountered a number of roadblocks. Whole-cell sensors have been limited by technological issues (e.g., plasmid loss and long response times [19,22]), concerns over biocontainment, and resource constraints associated with maintaining viability alongside complex genetic programs. CFPS-based sensors have exhibited variable sensitivity for different samples, sensitivity to **matrix effects** [25,26], and a loss of containment, protectivity, and certain sensing capabilities conferred by the cell membrane [19,25].

Leveraging attributes of both whole-cell and cell-free sensing, artificial cells may be well poised to advance biologically based sensing. While artificial cells can encapsulate enzymatic and strand-displacement systems [27–29], they are typically composed of a CFPS system and an encapsulating chassis, generally a bilayer membrane (Box 1). By re-introducing certain stabilizing features of the cellular membrane, particularly the ability to contain molecular machinery in the face of dilution [30] and protect against environmental components [31], artificial cells may reduce the impact of external conditions on the CFPS process, while posing a lower biohazard threat than living cells. Although a number of CFPS sensors have been developed that may function within artificial cells, the use of an encapsulating membrane as an active participant in sensing activities has seen limited progress. In particular, the incorporation of materials into the membrane that selectively permit certain signals, such as small-molecule analytes, to enter the artificial cell interior while retaining CFPS contents may significantly enhance sensing capabilities. As such, we expect membrane engineering to expand as a major factor in artificial cell design as it serves a critical role in recapitulating shielding and gating functions lost in the transition from whole-cell to CFPS sensing.

Top-down synthetic biology: an approach that aims to redirect the activities within living cells to generate new outputs and behaviors.
Vesicle: a spherical structure composed of a bilayer membrane surrounding an aqueous interior.
Whole-cell sensor: a top-down approach in which a living cell, often a bacterium or yeast, carries out engineered, genetically encoded behaviors in response to a specific signal using native expression machinery and cellular components.

Box 1. Assembling Membrane-Based Artificial Cells

Artificial cell chassis can be assembled from a variety of encapsulating materials, including coacervates [73], DNA–hydrogel compartments [60,61], and protein–polymer shells [27,46]. However, the majority of artificial cells to date have been assembled using bilayer membrane **vesicles** in the form of **liposomes** [33,34]. The use of a membrane-based chassis is often preferred due to its relative ease of assembly, compatibility with other biomolecules, and resemblance to the cell membrane. Alternative chassis materials, particularly hydrogels and protein-based structures [27,46,60,61], exhibit high mechanical stability and/or porosity suitable for protein diffusion, however, the enhanced control over features such as selective permeability and the incorporation of additional biological and synthetic components in liposomes has led to their widespread use in artificial cells. Paper-based systems have also been used to stabilize CFPS sensors, facilitating long-term storage and sensory assays in field settings, but like hydrogels and proteinosomes, lack the ability to incorporate membrane functions [6].

Assembly methods for liposomal artificial cells with encapsulated CFPS reactions typically derive from traditional methods to form **giant unilamellar vesicles**, including water-in-oil emulsions, thin film hydration, and microfluidics. Of these, emulsion phase transfer [74] and vesicle rehydration [75] are the most widely used for artificial cell sensor development [33,34]. While these techniques can generate gene-expressing liposome populations, each suffers from certain drawbacks. In particular, emulsion and microfluidic methods lack the ability to control membrane composition due to residual solvents or stabilizing surfactants that may stay in the membrane after vesicle formation [76,77], and thin film hydration methods exhibit poor encapsulation efficiency and generate heterogenous vesicle sizes and **lamellarities** [78]. Additionally, each demonstrated preparation method often results in heterogenous vesicle loading, with some vesicles exhibiting significantly higher protein expression than others [30,79]. Finally, while cellular membranes are composed of a large variety of lipids and biomolecules, artificial cells have, as of yet, not been recreated with this complexity [80]. Future work toward artificial cell development may require improvements in assembly methods that provide control over both vesicle physical properties and composition.

Functionalizing Synthetic Membranes through Composition Changes, Protein Insertion, and Surface Conjugation

As the primary boundary of the artificial cell, the chassis membrane serves a critical role in regulating interactions between an encapsulated CFPS sensor and its surrounding environment. Through membrane functionalization, a major focus in artificial cell sensor design is creating a balance in which the membrane can contain desired reactants while allowing the receipt of specific signals, often small molecules.

Membrane Composition Controls Stability, Permeability, and Membrane Protein Dynamics

Amphiphile selection provides one strategy to modulate properties of the artificial cell membrane (Figure 2A). These membranes have typically consisted of phosphatidylcholine (PC)-containing phospholipids, which are stable to modest changes in pH, temperature, and osmolarity, exhibit low phase transition temperatures, and are capable of self-assembly in the conditions suitable for CFPS [32–34]. Phospholipid membranes are semipermeable, allowing the passage of water and certain small molecules while excluding larger solutes [32]. The degree of permeability is dependent on membrane components and lipid-packing density, which can be tailored to balance the permeability of a desired analyte versus the leakage of encapsulated materials [35]. For example, permeability can be tuned by changing the length and degree of unsaturation of the phospholipids' hydrocarbon chains [32], by thermally inducing lipid-packing defects [36], or by incorporating components such as fatty acids [32,37] and cholesterol [38] into the membrane. Alternatively, certain synthetic materials such as polymers can be blended with phospholipid membranes to impart higher stability, lower permeability, and to facilitate the expression and spatially localized insertion of membrane proteins [31,39–42]. Polyethylene glycol (PEG) polymers can similarly be conjugated to lipid headgroups to modulate interactions at the membrane interface [43,44]. This ability to incorporate natural and synthetic materials into bilayer membranes offers enhanced control of membrane properties over what can be achieved in engineered live cells, providing an important handle to assemble robust artificial cell sensors.

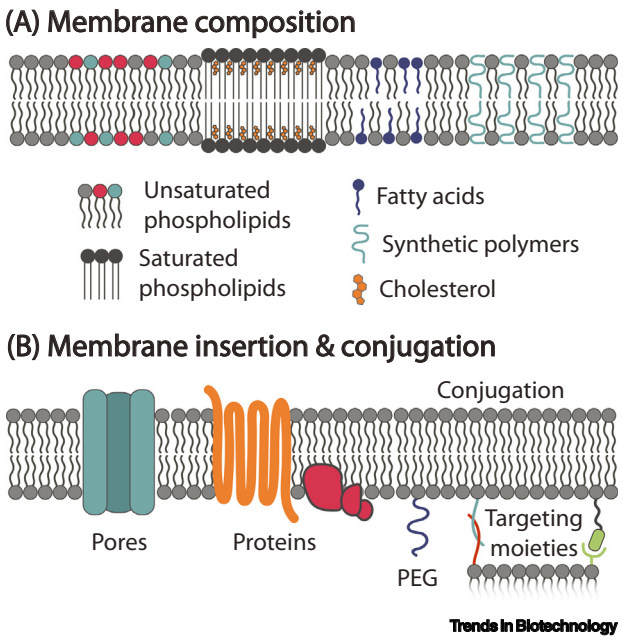

Figure 2. Membrane Functionalization through Changes in Composition and Biomolecular Insertion. (A) Membrane properties can be modulated by changing composition, for example, through the incorporation of phospholipids, cholesterol, fatty acids, and synthetic polymers. (B) Membrane activity can be altered by inserting or conjugating proteins, polymers, and other biomolecules into the membrane or onto its surface. Abbreviation: PEG, polyethylene glycol.

Membrane Proteins Transmit Environmental Signals

When compositional changes are insufficient to enable the detection of a specific signal, the incorporation of membrane proteins may be required (Figure 2B). To date, most artificial cell-based sensors for nonpermeable analytes have used simple pores to gate the entry or release of small molecules. In particular, the nonspecific, water-soluble pore α-hemolysin (αHL) has been widely explored in such systems [30,35,45–47]. αHL has been used to facilitate transport of small molecules up to 3 kDa into and out of artificial cells [47], enabling analyte entry [30,35], genetically regulated small-molecule release [30,45,46,48], and resupply of reactants from an external feeding solution [47]. While αHL incorporation provides a straightforward method to introduce small-molecule transport functions, a major tradeoff in its functionality is the leakage of encapsulated reactants. By contrast, many other membrane proteins control analyte gating with a high degree of specificity, which may offer an alternative method to tune artificial cell sensitivity and introduce increasingly complex functions into encapsulated sensing pathways.

To date, the incorporation of transmembrane proteins into naturally derived or synthetic vesicle membranes has been demonstrated for a select number of model proteins. While detergent reconstitution has been a popular method for membrane protein incorporation, the past decade has seen the expansion of CFPS methods to cotranslationally integrate membrane proteins into vesicle membranes [41,49–51]. Several proteins have been integrated into vesicles in this way; this includes large protein complexes like ATP synthase [52] as well as various membrane receptors, including G-protein-coupled receptors [53–55]. There are many membrane proteins left to explore and much left to uncover regarding the effects of membrane composition on proper folding and activity of membrane proteins that are cotranslationally inserted into membranes [49]. The level of success exploring these relationships so far is promising, however, and a better understanding of the design rules to incorporate a wider range of membrane proteins will expand the repertoire of behaviors that are possible in artificial cell systems.

Membrane Functionalization Can Mediate Encapsulated Cell-Free Reactions

Membrane properties not only impact signaling but can provide a route to further enhance or control encapsulated CFPS reactions. Pore incorporation and enzymatic reactions can be spatially localized to specific structures within a larger vesicle by creating nested vesicle-in-vesicle structures with distinct membrane compositions, much like cellular organelles [56]. Alternatively, the insertion of SNARE protein mimics and the conjugation of cDNA oligos to the vesicle membrane can control targeting and fusion between populations of vesicles (Figure 2B) [30,57]. This barcoding functionality provides a route to deliver genetic information, initiate and modulate genetically encoded reactions, and control the sequence of fusion events between specific populations of vesicles to facilitate complex, multistep reactions [30,57,58]. Finally, membrane-localized PEG molecules can be harnessed to enhance encapsulated CFPS reactions and direct spatially localized protein assembly [43,44]. For sensing applications, in which the retention and activity of encapsulated CFPS components must be balanced with the receipt and processing of new environmental information, these types of membrane functionalization strategies to spatially and temporally control CFPS may expand the possibilities to tailor application-specific sensor platforms.

Artificial Cells Can Sense Small Molecules, Mechanical Forces, and Bacterial Signals

Once assembled through the combination of an appropriate CFPS system and corresponding membrane components, the artificial cell can be harnessed for sensing. Artificial cell sensing uses genetic circuits to translate a signal into a detectable output, which has primarily been accomplished through membrane gating (Figure 3A), encapsulation of signal-responsive CFPS

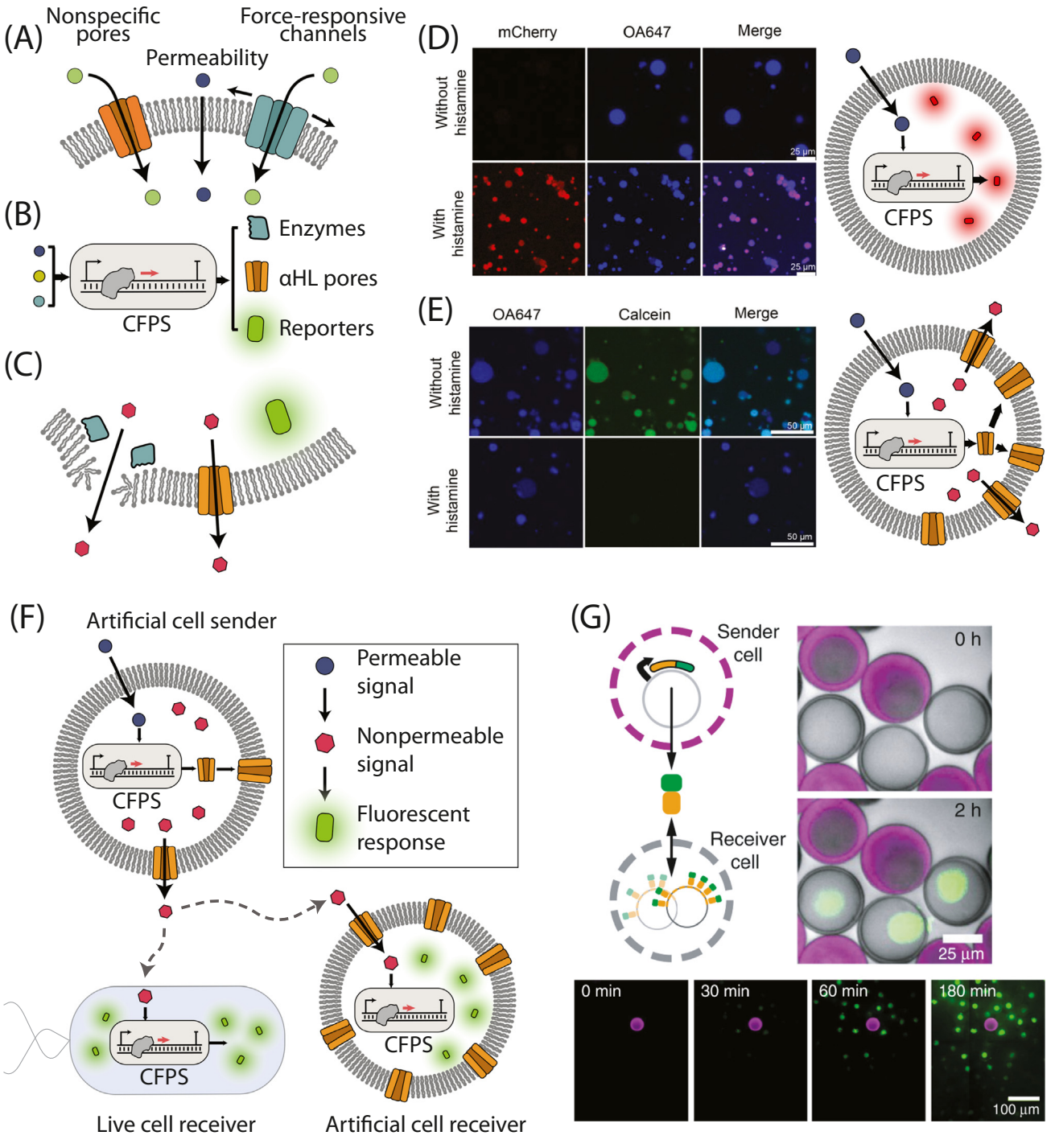

Figure 3. Cell-Free Sensor Functionality. (A) Signal detection is the first step in sensing, which has been achieved in artificial cells through permeability-regulated diffusion, nonspecific channels, and specific force and light-responsive membrane proteins. (B) Once an analyte enters the artificial cell, the initiation of a cell-free protein synthesis (CFPS) reaction leads to an observable output, often expression of an enzyme, pore, or reporter protein such as GFP. (C) The cell-free production of these products generates an artificial cell response, often fluorescent signal retention, content release through pores, or membrane lysis. (D) mCherry protein

(Figure legend continued at the bottom of the next page.)

systems (Figure 3B), and design of genetically encoded outputs, varying from reporter expression to membrane lysis (Figure 3C). Recent studies have highlighted how these factors can be combined to develop stimuli-responsive artificial cells that serve as small molecule indicators, chemical translators, light sensors, and force sensors (Table 1).

Sensing Molecular Signals and Engineering Small-Molecule-Based Communication

A major goal in biosensing is the ability to report the presence of specific small molecule signals. Toward this, artificial cell sensing for small molecules has focused on expanding the types of signals that can be detected, as well as incorporating genetically programmed outputs that can be initiated upon analyte detection. One focus of this work has been the development of artificial cells that sense membrane-permeable signals [30,35,45,59] to induce the expression of products including reporter proteins (Figure 3D) [30,45], pore molecules (Figure 3E) [30,45,59], and enzymes [45]. Membrane-permeable signals are straightforward for these applications in that no transporters are required for analyte entry, allowing the membrane barrier to remain largely intact. By contrast, αHL has been an effective mechanism to detect molecules that are large, polar, or otherwise unable to diffuse across the membrane [30,35], but artificial cells generally need to be kept within a feeding solution to compensate for reactant loss [47]. While these strategies have each resulted in artificial cells capable of sensing various small-molecule signals, it is the combinatorial use of these approaches that has been powerful in creating new sensing pathways. The detection of permeable molecules can initiate the expression of αHL pores, allowing controlled release of impermeable cargo in response to a permeable signal. This can serve as a functional response itself but can also be used to send signals to other cells, living or artificial. For example, this process has been used to translate an otherwise unrecognizable molecule for *Escherichia coli* into a native signal [59], and to send signals between different populations of αHL-functionalized artificial cells [30] (Figure 3F). The development of artificial cells that respond to permeable and impermeable molecules has allowed for the creation of sensors that can detect small molecule signals and serve as sensing intermediates, and has facilitated the development of new stepwise signaling pathways, establishing population-specific responses [30] and circumventing the need to directly engineer live bacteria [59].

While most artificial cell sensors for small molecules have used bilayer membranes in some capacity, non-membranous compartments have proven useful as vesicle-interfacing and standalone sensors as well. Proteinosomes, structures composed of protein–polymer conjugates, can exhibit enzymatic functionality that allows them to communicate chemically with αHL-expressing liposomes [46]. Alternatively, hydrogel compartments, which are more permeable and osmotically robust than their lipid counterparts, are well-suited for sensing pathways involving larger molecules, such as proteins [60,61] (Figure 3G). While these hydrogel platforms exhibit improved mechanical stability compared with liposome-based artificial cells [60], their increased porosity, which enables protein-based signaling, also significantly reduces the retention of CFPS reactants. The enhanced robustness of hydrogels or the enzymatic activity of proteinosomes may be advantageous over liposomes if selective permeability is noncritical and feeding solutions can be maintained. Liposomes, by contrast, offer improved abilities to selectively engineer chassis permeability to retain CFPS reactants and to incorporate diverse membrane proteins and channels. While

expression is observed in response to the diffusion of histamine into artificial cells, correlated with an encapsulated volume marker, OA647. Reprinted, with permission, from [45]. (E) Content release through expressed, α hemolysin (αHL) pores is observed in response to histamine diffusion into artificial cells. Reprinted, with permission, from [45]. (F) Artificial cells communicate through small molecule sensing and release. An artificial cell receiving a permeable signal expresses αHL pores, leading to the release of an impermeable cargo. This impermeable cargo is detected by either a live cell or another artificial cell nearby, which then generates an observable response. (G) Protein-based signaling is observed in artificial cells with porous polymer membranes. A sender cell produces a tagged fluorescent protein, which binds to DNA in a receiver's hydrogel nucleus and generates a cell-type-specific response through accumulation. Protein diffusion can be observed spatially over time, leading to more dispersed signaling as time increases. Reprinted, with permission, from [60].

Table 1. Artificial Cells with Sensing Behaviors[a]

Signals	Mode of entry	Artificial cell platform; liposome composition	Communication	Output	Refs
Theophylline; IPTG	Diffusion; αHL	Liposome; POPC, cholesterol	With *Escherichia coli*	Translation of permeable to impermeable signal via αHL expression	[59]
Arabinose, theophylline; IPTG, doxycycline	Diffusion; αHL	Liposome; POPC, cholesterol	Between artificial cell populations	Translation of permeable to impermeable signal via αHL expression; spatial segregation of transcription and translation processes	[30]
Histamine	Diffusion	Liposome; EggPC, cholesterol	N/A	Expression of reporter protein, lytic enzyme, or αHL pore	[45]
3OC6HSL, DFHBI, IPTG, aTc; arabinose, rhamnose, DAPG, guanine	Diffusion; αHL	Lipid-based water-in-oil droplets; DOPC, DOPG, DPhPG, Cholesterol	Between spatially defined artificial cell populations	Diffusion range sensor, feed-forward circuit, positive-feedback circuit	[35]
3OC6HSL; Glucose	Diffusion	Proteinosome and liposome; POPC, cholesterol	Between liposomes and proteinosomes	Translation of permeable to impermeable signal via αHL expression; glucose release leading to fluorescent output in proteinosomes	[46]
TetR-sfGFP, TetR-mCherry, T3 RNA Polymerase	Diffusion	Polymersome and clay–DNA hydrogel	Between artificial cell populations	Reporter protein expression	[60]
IPTG	Diffusion	Aptamer-grafted hydrogel	N/A	Reporter protein expression	[61]
Osmotic pressure, Ca^{2+}	MscL	Liposome; EggPC, DOPC, cholesterol	N/A	Reporter protein expression	[63]
Osmotic pressure, IPTG	MscL	Liposome; DOPC, DOPE, cholesterol	N/A	Cytoskeletal protein (MreB) expression	[62]
Ca^{2+}, lipid catalysis	αHL, MscL	Liposome; DOPC, DOPG, POPC	N/A	Dye release	[56]
Light	Photolabile DNA cage	Liposome; DMPC	N/A	Reporter protein and enzyme expression	[64]
Light	ATP synthase, bacterio-rhodopsin	Liposome; POPC, cholesterol	N/A	Bacteriorhodopsin expression in response to light-stimulated ATP generation	[65]
3OC6HSL, IPTG	Diffusion	Surfactant-based water-in-oil droplets	With *E. coli*	AHL detection, production and release; IPTG AND gate	[67]
Various bacterial AHLs	Diffusion	Liposome; POPC, cholesterol	With *Vibrio fischeri*, *Vibrio harveyi,* and *E. coli*	Reporter protein expression; AHL detection, production, and release	[68]
3OC6HSL	Diffusion	Liposome; DOPC, POPC, EggPC, cholesterol	With *E. coli*	Reporter protein expression; AHL detection, production, and release; Environmental conditions	[69]
3OC6HSL	Perfringolysin O pores (PFO)	Liposome; POPC, cholesterol	Between artificial cells and neural stem cells	PFO expression in response to 3OC6HSL, subsequent release of BDNF leading to neural differentiation; Physiological conditions	[70]

[a]Abbreviations: 3OC6HSL, *N*-(3-oxo-hexanoyl)- L-homoserine lactone (bacterial AHL); aTc, anhydrotetracycline; DAPG, 2,4-diacetylphloroglucinol; DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphocholine (lipid); DOPC, 1,2-dioleoyl-sn-glycero-3-phosphocholine (lipid); DOPE, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (lipid); DOPG, 1,2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (lipid); DPhPG, 1,2-diphytanoyl-sn-glycero-3-phosphocholine (lipid); EggPC, L-α-phosphatidylcholine (lipid); IPTG, isopropyl β-D-1-thiogalactopyranoside; POPC, 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (lipid); TetR-mCherry, fusion protein of TetR and mCherry fluorescent protein; TetR-sfGFP, fusion protein of tetracycline repressor (TetR) and superfolder GFP.

liposomes have been more widely used for artificial cell development to date, non-membranous artificial cells that have new material properties may be better suited to specific applications yet to be explored.

Artificial Cells Expand the Capabilities of Cell-Free Sensing for Mechanical Forces and Light

Within a dynamic environment, small molecule signals are not the only stimuli present. For example, signals such as physical force or light intensity may be important contextual clues for environmental monitoring. With this in mind, the development of mechanosensitive and light-

sensitive artificial cells are powerful examples of how membrane engineering can expand the sensing capabilities of CFPS systems.

In order to develop force-sensitive artificial cells, one approach has been the incorporation of mechanically sensitive membrane proteins, such as the *E. coli* mechanosensitive channel of large conductance (MscL). Originally an osmotically activated release valve in bacteria, MscL can be expressed into artificial cell membranes using CFPS, creating a mechanosensitive gate which opens in response to membrane stretching [41,56,62,63]. MscL in artificial cells has been shown to open to form a 3-nm pore, allowing an influx of small molecule inducers into the artificial cell interior to initiate encapsulated CFPS reactions in an AND gate fashion [56,62,63]. This has been demonstrated to occur in response to both osmotic stress [62,63] as well as enzymatically induced changes in lateral membrane pressure [56]. MscL function in these platforms has been coupled to protein expression, including fluorescent reporter expression [63] and cytoskeletal protein expression and assembly [62], as well as controlled content release [56]. The role of membrane composition remains important here, as recent work by Hindley and colleagues demonstrated that, through the creation of a vesicle-in-vesicle superstructure with distinct compositions and protein incorporation, MscL could respond to membrane morphological changes to allow the release of a fluorescent dye from the innermost vesicles [56]. By harnessing the natural function of MscL for use in artificial cell settings, these studies illustrate how the incorporation of a specific membrane protein can expand force sensing capabilities in dynamic environments.

In addition to mechanical force, artificial cells have been developed that express protein products in response to light stimuli. This has been accomplished through both CFPS and membrane-based methods, including the incorporation of light-cleavable protecting groups on CFPS DNA [64], and the functional incorporation of ATP synthase and bacteriorhodopsin membrane proteins [65]. Importantly, the latter approach capitalized on naturally existing photosynthetic pathways to drive protein synthesis in a positive feedback loop, harnessing energy generation to drive further membrane protein expression. This use of specialized light-sensitive membrane proteins, in particular, highlights an exciting step toward artificial cells that are capable of recapitulating certain sensing features that have, until now, been unique to living cells.

Artificial Cells to Infiltrate, Monitor, and Modulate Bacterial Communities

With the demonstrated ability to sense, report, and respond to biological signals, artificial cells present an opportunity to infiltrate and interact with communities of live cells in order to direct cellular behavior (Figure 3F). A particular focus in this regard has been the use of artificial cells to interact with communities of live bacteria through quorum sensing molecules, which serve as indicators of cell density and play important roles in cooperative processes such as biofilm formation [66]. These quorum-sensing molecules – especially **acylated homoserine lactones (AHLs)**, which readily diffuse through lipid membranes – have proven to be a useful handle to allow artificial cells to communicate with live bacteria [59,67,68]. Artificial cells have been designed not only to respond to AHLs received from bacteria but to serve as actuators of live cell behavior by synthesizing and releasing bacteria-specific AHLs. This has been demonstrated both within populations of a single type of bacteria, specifically *E coli.* [67], as well as between different bacterial species [59,68]. Highlighting the modularity of these strain-specific AHL circuits, Lentini and colleagues recently explored the use of various AHLs in artificial cells to communicate with four different species of bacteria: *Vibrio fischeri*, *Vibrio harveyi*, *E. coli*, and *Pseudomonas aeruginosa* [68]. By combining genetic instructions to detect an AHL from one bacterial species and release an AHL for another, they created a new communication pathway between incompatible bacterial species with artificial cells as a sensing intermediate. They also demonstrated the ability to inhibit

signaling by designing artificial cells that released an enzyme to break down *P. aeruginosa* AHLs when *V. fischeri* was present, disrupting quorum sensing altogether. This ability to combine genetically encoded instructions for different quorum sensing molecules with separate AHL outputs shows how artificial cells can serve as a checkpoint for cell–cell interactions, leading to new pathways that enable artificial cells to hijack or sever bacterial communication.

Moving from the Laboratory to the Field

To date, artificial cells show exciting promise in the development of self-contained biological sensors. However, many of these proof-of-concept studies have been conducted in highly controlled conditions to maintain stability, function, and cell viability. Real-world applications may present new constraints associated with naturally occurring conditions, which could differ considerably from laboratory conditions. Ding and colleagues recently investigated the consequences of variable environmental conditions on the function of artificial cell sensors [69], finding significant improvement in the performance of encapsulated quorum sensing networks upon optimizing artificial cells to overcome osmotic imbalances, increase molecular crowding, and improve membrane stability. Similar considerations arise when creating interfaces with eukaryotic cells in physiological conditions, particularly with maintaining the viability of living cells. To address this challenge, Toparlak and colleagues engineered artificial cells which could express a non-specific pore, perfringolysin O (PFO), to release brain-derived neurotropic factor (BDNF) in response to an AHL signal [70]. By optimizing a CFPS system for low toxicity and physiological osmolarity, artificial cells could produce and release protein signals in the presence of live neural stem cells, ultimately stimulating cellular differentiation. While artificial cell sensors have yet to be widely deployed in many sensing contexts, these studies demonstrate factors that are likely to become increasingly important in artificial cell design and give insight into future modifications that will be necessary to achieve this goal.

Concluding Remarks and Future Perspectives

As the technologies of cell-free sensing and membrane engineering converge, the development of stimuli-responsive artificial cells is rapidly expanding. While significant progress has been made toward artificial cells that can sense environmental signals, critical limitations include a small number of analytes that can currently be detected, a lack of physical stability, and poor balance between membrane permeability and reaction retention. In order to address these issues, it is likely that techniques to assemble multicomponent artificial membranes, which confer stability and molecular gating, and CFPS sensing systems that detect diverse analytes will need to expand in parallel, with new strategies to create more dynamic interfaces between the two. An increasing number of analytes may be detectable through the incorporation of additional protein or nucleic acid-based sensing strategies that do not rely on gene expression. These modules offer temporal improvement over the hours-long response times characteristic of genetically encoded systems [27,29]. Analyte transport may be further modulated through membrane compositional changes or through fusion-based reagent delivery in order to introduce small molecules to the artificial cell interior without suffering reactant loss. Finally, structural components such as artificial cytoskeletons may help push artificial cells closer to whole-cell robustness [62]. With the various components that can be incorporated into these sensor platforms, it is possible that artificial cells may ultimately be applicable in many different biosensing fields. In particular, applications that require monitoring of aqueous systems, such as environmental remediation, agriculture, and *in vivo* sensing [70,71], may be the first to see field-applicable artificial cell sensors (Figure 4). This is primarily a result of the biological nature of cell-free systems, which inherently require an aqueous environment to function, as well as the stability of self-assembling chassis materials in aqueous conditions. Moving forward, sensing in non-aqueous environments may be facilitated by using new materials that interface aqueous and organic environments [72].

Outstanding Questions

What technological improvements need to occur in order to assemble artificial cells with complex membrane compositions and functionalization? How do we improve encapsulation efficiency, unilamellarity, size distribution, and compositional control while retaining all components of CFPSs and their activity?

Artificial membranes to date are much less diverse in composition than naturally occurring cell membranes; how does increasing the complexity of artificial membranes affect their designed activities?

How do existing limitations in CFPS sensor encapsulation, particularly unequal vesicle loading and small-molecule loss, impact the reliability of artificial cell-based sensors?

How do CFPS components interact with artificial membranes? How does this interaction impact the resulting sensing behavior as well as artificial cell stability and functionality?

How do we incorporate complex membrane proteins in synthetic systems to facilitate sensing behaviors? What considerations are most important (e.g., membrane composition, artificial cell size, insertion method, protein features, and/or protein orientation)?

What properties does an artificial cell need to exhibit in order to serve as a functional sensor in real-world applications? How do we robustly transition this technology from laboratory-based settings to field-based applications?

How stable are artificial cells long-term? How do we improve shelf-life to reduce the limitations in artificial cell application?

What tools, containment strategies, and analysis methods are necessary for the functional use of artificial cell sensors?

What ethical concerns arise in the development and deployment of artificial cells? How does this compare with other biologically based sensors, such as whole-cell and cell-free sensors? What regulatory considerations need to be considered in order to address these concerns?

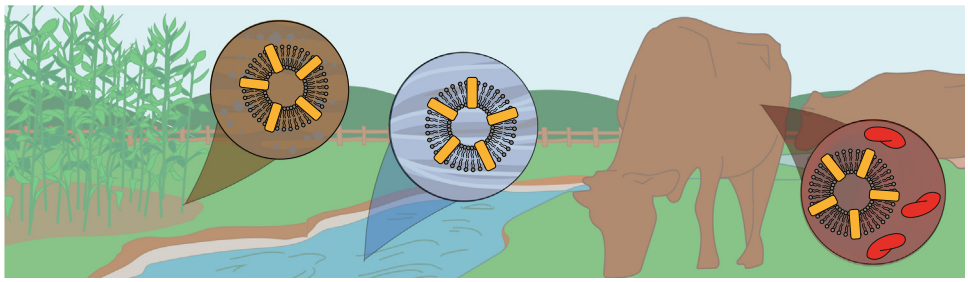

Figure 4. Outlook of Artificial Cell Sensing. Artificial cells may be useful platforms for biosensing in a number of contexts, particularly in aqueous environments. For example, they may be useful for monitoring soil in agricultural applications, for monitoring water quality, or even for use as *in vivo* biosensors in live organisms such as livestock. While membrane transport remains a hurdle to be addressed, artificial cells may eventually be able to detect pesticides, contaminants, bacteria, disease markers, and other important signals in a number of environments.

The examples highlighted here represent exciting steps toward the use of artificial cells for a number of complex, analyte-responsive behaviors, which range from simple sensors to actuators of live cell behavior. With the limited applications to date, however, artificial cells have yet to realize the breadth of possibilities for both encapsulated CFPS sensors as well as membrane functionalization strategies (see Outstanding Questions). In part, this is because the assembly of artificial cells involves complex interactions between a large number of molecules, a system that can be particularly hard to troubleshoot and optimize. Additionally, the existing toolbox of available and compatible natural and synthetic components – although expanding – is still limited compared with that found in nature. As a result, significant work remains to fully characterize both the artificial systems and the natural cells that inspire their design. In particular, artificial cell capabilities could be expanded through a better understanding of CFPS/chassis interactions, methods to improve loading efficiency, and membrane protein design and incorporation rules. Importantly, these investigations will be informed by ongoing characterization of cellular membrane properties and identification of additional intracellular sensing pathways in living cells. Together, these strategies could ultimately identify and isolate additional cell-inspired sensing modalities while better characterizing the biophysical and biochemical properties of artificial cell assemblies, furthering the transition to diverse, robust, and technologically viable biosensing platforms. While we are yet unable to recapitulate the complexities of a living cell, we are consistently working toward a better understanding of the pathways that allow them to monitor and respond to their environment. Eventually artificial cells may be able to receive and process a number of inputs into complex outputs, much like live cells, in order to build user-defined, biologically based systems to monitor the world around us.

Acknowledgments

The authors thank Dr Joshua Kogot and members of the Kamat laboratory for helpful discussions. M.A.B. received funding from the Department of Defense through the National Defense Science and Engineering Graduate Fellowship. This work was supported in part by the Air Force Office of Scientific Research (AFOSR) FA9550-19-1-0039 award to N.P.K and the National Science Foundation (NSF) under Grant No. 1844336 and Grant No. 1844219.

References

1. Shi, S. *et al.* (2018) *In vivo* biosensors: mechanisms, development, and applications. *J. Ind. Microbiol. Biotechnol.* 45, 491–516
2. Morales-Narváez, E. and Dincer, C. (2020) The impact of biosensing in a pandemic outbreak: COVID-19. *Biosens. Bioelectron.* 163, 112274
3. Mollarasouli *et al.* (2019) The role of electrochemical immunosensors in clinical analysis. *Biosensors* 9, 86
4. Dai, Y. and Liu, C.C. (2019) Recent advances on electrochemical biosensing strategies toward universal point-of-care systems. *Angew. Chem.* 131, 12483–12496
5. Slomovic, S. *et al.* (2015) Synthetic biology devices for *in vitro* and *in vivo* diagnostics. *Proc. Natl. Acad. Sci. U. S. A.* 112, 14429–14435
6. Pardee, K. *et al.* (2014) Paper-based synthetic gene networks. *Cell* 159, 940–954
7. Pardee, K. *et al.* (2016) Rapid, low-cost detection of Zika virus using programmable biomolecular components. *Cell* 165, 1255–1266
8. Riangrungroj, P. *et al.* (2019) A label-free optical whole-cell *Escherichia coli* biosensor for the detection of pyrethroid insecticide exposure. *Sci. Rep.* 9, 1–9

9. Miller, R.A. *et al.* (2020) Development of a paper-immobilized yeast biosensor for the detection of physiological concentrations of doxycycline in technology-limited settings. *Anal. Methods* 12, 2123–2132
10. Jia, X. *et al.* (2019) Sensitive and specific whole-cell biosensor for arsenic detection. *Appl. Environ. Microbiol.* 85, e00694-19
11. Mimee, M. *et al.* (2018) An ingestible bacterial-electronic system to monitor gastrointestinal health. *Science* 360, 915–918
12. Lin, C. *et al.* (2019) Development of a whole-cell biosensor for the determination of tyrosine in urine for point-of-care diagnostics. *Anal. Methods* 11, 1400–1404
13. Jia, X. *et al.* (2018) Gene circuit engineering to improve the performance of a whole-cell lead biosensor. *FEMS Microbiol. Lett.* 365, fny157
14. Watstein, D.M. and Styczynski, M.P. (2018) Development of a pigment-based whole-cell zinc biosensor for human serum. *ACS Synth. Biol.* 7, 267–275
15. Mao, N. *et al.* (2018) Probiotic strains detect and suppress cholera in mice. *Sci. Transl. Med.* 10, eaao2586
16. Ma, D. *et al.* (2018) Low-cost detection of norovirus using paper-based cell-free systems and synbody-based viral enrichment. *Synth. Biol.* 3, ysy018
17. Verosloff, M. *et al.* (2019) PLANT-Dx: a molecular diagnostic for point-of-use detection of plant pathogens. *ACS Synth. Biol.* 8, 902–905
18. Zhang, P. *et al.* (2019) Detection of inorganic ions and organic molecules with cell-free biosensing systems. *J. Biotechnol.* 300, 78–86
19. Gräwe, A. *et al.* (2019) A paper-based, cell-free biosensor system for the detection of heavy metals and date rape drugs. *PLoS One* 14, e0210940
20. Thavarajah, W. *et al.* (2020) Point-of-use detection of environmental fluoride via a cell-free riboswitch-based biosensor. *ACS Synth. Biol.* 9, 10–18
21. Liu, X. *et al.* (2020) Design of a transcriptional biosensor for the portable, on-demand detection of cyanuric acid. *ACS Synth. Biol.* 9, 84–94
22. Silverman, A.D. *et al.* (2020) Design and optimization of a cell-free atrazine biosensor. *ACS Synth. Biol.* 9, 671–677
23. Takahashi, M.K. *et al.* (2018) A low-cost paper-based synthetic biology platform for analyzing gut microbiota and host biomarkers. *Nat. Commun.* 9, 1–12
24. Wen, K.Y. *et al.* (2017) A cell-free biosensor for detecting quorum sensing molecules in *P. aeruginosa*-infected respiratory samples. *ACS Synth. Biol.* 6, 2293–2301
25. McNerney, M.P. *et al.* (2019) Point-of-care biomarker quantification enabled by sample-specific calibration. *Sci. Adv.* 5, eaax4473
26. Voyvodic, P.L. *et al.* (2019) Plug-and-play metabolic transducers expand the chemical detection space of cell-free biosensors. *Nat. Commun.* 10, 1697
27. Joesaar, A. *et al.* (2019) DNA-based communication in populations of synthetic protocells. *Nat. Nanotechnol.* 14, 369–378
28. Hindley, J.W. *et al.* (2018) Light-triggered enzymatic reactions in nested vesicle reactors. *Nat. Commun.* 9, 1093
29. Peng, R. *et al.* (2020) DNA-based artificial molecular signaling system that mimics basic elements of reception and response. *Nat. Commun.* 11, 978
30. Adamala, K.P. *et al.* (2017) Engineering genetic circuit interactions within and between synthetic minimal cells. *Nat. Chem.* 9, 431–439
31. Meyer, C.E. *et al.* (2020) Biomolecule–polymer hybrid compartments: combining the best of both worlds. *Phys. Chem. Chem. Phys.* 22, 11197–11218
32. Monnard, P.-A. and Deamer, D.W. (2002) Membrane self-assembly processes: steps toward the first cellular life. *Anat. Rec.* 268, 196–207
33. Hindley, J.W. *et al.* (2020) Membrane functionalization in artificial cell engineering. *SN Appl. Sci.* 2, 1–10
34. Stano, P. (2019) Gene expression inside liposomes: from early studies to current protocols. *Chem. A Eur. J.* 25, 7798–7814
35. Dupin, A. and Simmel, F.C. (2019) Signalling and differentiation in emulsion-based multi-compartmentalized *in vitro* gene circuits. *Nat. Chem.* 11, 32–39
36. Monnard, P.-A. *et al.* (2007) Models of primitive cellular life: polymerases and templates in liposomes. *Philos. Trans. R. Soc. B Biol. Sci.* 362, 1741–1750
37. Mansy, S.S. *et al.* (2008) Template-directed synthesis of a genetic polymer in a model protocell. *Nature* 454, 122–125
38. Zocher, F. *et al.* (2013) Local partition coefficients govern solute permeability of cholesterol-containing membranes. *Biophys. J.* 105, 2760–2770
39. Discher, D.E. and Eisenberg, A. (2000) Polymer vesicles. *J. Colloid Interface Sci.* 290, 525
40. Petit, J. *et al.* (2018) A modular approach for multifunctional polymersomes with controlled adhesive properties. *Soft Matter* 14, 894–900
41. Jacobs, M.L. *et al.* (2019) Diblock copolymers enhance folding of a mechanosensitive membrane protein during cell-free expression. *Proc. Natl. Acad. Sci.* 116, 4031–4036
42. Kowal, J. *et al.* (2015) Hybrid polymer-lipid films as platforms for directed membrane protein insertion. *Langmuir* 31, 4868–4877
43. Garenne, D. *et al.* (2020) Membrane molecular crowding enhances MreB polymerization to shape synthetic cells from spheres to rods. *Proc. Natl. Acad. Sci. U. S. A.* 117, 1902–1909
44. Garenne, D. and Noireaux, V. (2020) Analysis of cytoplasmic and membrane molecular crowding in genetically programmed synthetic cells. *Biomacromolecules* 21, 2808–2817
45. Dwidar, M. *et al.* (2019) Programmable artificial cells using histamine-responsive synthetic riboswitch. *J. Am. Chem. Soc.* 141, 11103–11114
46. Tang, t.-y.d. *et al.* (2018) gene-mediated chemical communication in synthetic protocell communities. *ACS Synth. Biol.* 7, 339–346
47. Noireaux, V. and Libchaber, A. (2004) A vesicle bioreactor as a step toward an artificial cell assembly. *Proc. Natl. Acad. Sci.* 101, 17669–17674
48. Hilburger, C.E. *et al.* (2019) Controlling secretion in artificial cells with a membrane and gate. *ACS Synth. Biol.* 8, 1224–1230
49. Henrich, E. *et al.* (2015) Membrane protein production in *Escherichia coli* cell-free lysates. *FEBS Lett.* 589, 1713–1722
50. Hovijitra, N.T. *et al.* (2009) Cell-free synthesis of functional aquaporin Z in synthetic liposomes. *Biotechnol. Bioeng.* 104, 40–49
51. Uyeda, A. *et al.* (2016) Construction of an *in vitro* gene screening system of the *E. coli* EmrE transporter using liposome display. *Anal. Chem.* 88, 12028–12035
52. Matthies, D. *et al.* (2011) Cell-free expression and assembly of ATP synthase. *J. Mol. Biol.* 413, 593–603
53. Hamada, S. *et al.* (2014) Giant vesicles functionally expressing membrane receptors for an insect pheromone. *Chem. Commun.* 50, 2958–2961
54. Fenz, S.F. *et al.* (2014) Cell-free synthesis of membrane proteins: tailored cell models out of microsomes. *Biochim. Biophys. Acta Biomembr.* 1838, 1382–1388
55. Sonnabend, A. *et al.* (2017) Production of G protein-coupled receptors in an insect-based cell-free system. *Biotechnol. Bioeng.* 114, 2328–2338
56. Hindley, J.W. *et al.* (2019) Building a synthetic mechanosensitive signaling pathway in compartmentalized artificial cells. *Proc. Natl. Acad. Sci. U. S. A.* 116, 16711–16716
57. Peruzzi, J.A. *et al.* (2019) Barcoding biological reactions with DNA-functionalized vesicles. *Angew. Chem.* 131, 18856–18863
58. Gaut, N.J. *et al.* (2019) Differentiation of pluripotent synthetic minimal cells via genetic circuits and programmable mating. *bioRxiv* Published online July 24, 2019. https://doi.org/10.1101/712968
59. Lentini, R. *et al.* (2014) Integrating artificial with natural cells to translate chemical messages that direct *E. coli* behaviour. *Nat. Commun.* 5, 4012
60. Niederholtmeyer, H. *et al.* (2018) Communication and quorum sensing in non-living mimics of eukaryotic cells. *Nat. Commun.* 9, 1–8
61. Lai, S.N. *et al.* (2020) Artificial cells capable of long-lived protein synthesis by using aptamer grafted polymer hydrogel. *ACS Synth. Biol.* 9, 76–83
62. Garamella, J. *et al.* (2019) An adaptive synthetic cell based on mechanosensing, biosensing, and inducible gene circuits. *ACS Synth. Biol.* 8, 1913–1920
63. Majumder, S. *et al.* (2017) Cell-sized mechanosensitive and biosensing compartment programmed with DNA. *Chem. Commun.* 53, 7349–7352
64. Schroeder, A. *et al.* (2012) Remotely activated protein-producing nanoparticles. *Nano Lett.* 12, 2685–2689
65. Berhanu, S. *et al.* (2019) Artificial photosynthetic cell producing energy for protein synthesis. *Nat. Commun.* 10, 1325

66. Miller, M.B. and Bassler, B.L. (2001) Quorum sensing in bacteria. *Annu. Rev. Microbiol.* 55, 165–199
67. Schwarz-Schilling, M. *et al.* (2016) Chemical communication between bacteria and cell-free gene expression systems within linear chains of emulsion droplets. *Integr. Biol.* 8, 564–570
68. Lentini, R. *et al.* (2017) Two-way chemical communication between artificial and natural cells. *ACS Cent. Sci.* 3, 117–123
69. Ding, Y. *et al.* (2018) Minimizing context dependency of gene networks using artificial cells. *ACS Appl. Mater. Interfaces* 10, 30137–30146
70. Toparlak, Ö.D. *et al.* (2020) Artificial cells drive neural differentiation. *Sci. Adv.* 6, eabb4920
71. Krinsky, N. *et al.* (2018) Synthetic cells synthesize therapeutic proteins inside tumors. *Adv. Healthc. Mater.* 7, 1701163
72. Panganiban, B. *et al.* (2018) Random heteropolymers preserve protein function in foreign environments. *Science* 359, 1239–1243
73. Tian, L. *et al.* (2019) Artificial morphogen-mediated differentiation in synthetic protocells. *Nat. Commun.* 10, 3321
74. Pautot, S. *et al.* (2003) Production of unilamellar vesicles using an inverted emulsion. *Langmuir* 19, 2870–2879
75. Spencer, A.C. *et al.* (2013) The encapsulation of cell-free transcription and translation machinery in vesicles for the construction of cellular mimics. *J. Vis. Exp.* 80, e51304
76. Kamat, N.P. *et al.* (2011) Micropipette aspiration of double emulsion-templated polymersomes. *Soft Matter* 7, 9863–9866
77. Deshpande, S. *et al.* (2016) Octanol-assisted liposome assembly on chip. *Nat. Commun.* 7, 10447
78. Nele, V. *et al.* (2019) Effect of formulation method, lipid composition, and PEGylation on vesicle lamellarity: a small-angle neutron scattering study. *Langmuir* 35, 6064–6074
79. Sakamoto, R. *et al.* (2018) Anomalous scaling of gene expression in confined cell-free reactions. *Sci. Rep.* 8, 1–8
80. Garenne, D. and Noireaux, V. (2020) Membrane functions genetically programmed in synthetic cells: a barrier to conquer. *Curr. Opin. Syst. Biol.* 24, 9–17