15. Schricker, S., Palacio, M., Thirumamagal, B.T., Bhushan, B.: Synthesis and morphological characterization of block copolymers for improved biomaterials. Ultramicroscopy **110**, 639–649 (2010)
16. Palacio, M., Schricker, S., Bhushan, B.: Bioadhesion of various proteins on random, diblock, and triblock copolymer surfaces and the effect of pH conditions. J.R. Soc. Interface **8**, 630–640 (2011)
17. George, P.A., Donose, B.C., Cooper-White, J.J.: Self-assembling polystyrene-block-poly(ethylene oxide) copolymer surface coatings: resistance to protein and cell adhesion. Biomaterials **30**, 2449–2456 (2009)
18. George, P.A., Doran, M.R., Croll, T.I., Munro, T.P., Cooper-White, J.J.: Nanoscale presentation of cell adhesive molecules via block copolymer self-assembly. Biomaterials **30**, 4732–4737 (2009)

Biocalorimetry

► Nanocalorimetry

Bioderived Smart Materials

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Definition

Bioderived smart materials are a category of ionic active materials that are fabricated from biological macromolecules and utilize the ion transport properties of cell membranes to couple multiple physical domains (chemical, electrical, mechanical, and optical). Owing to this coupling exhibited by biological macromolecules, bioderived smart materials are used as actuators, sensors, and energy harvesting applications.

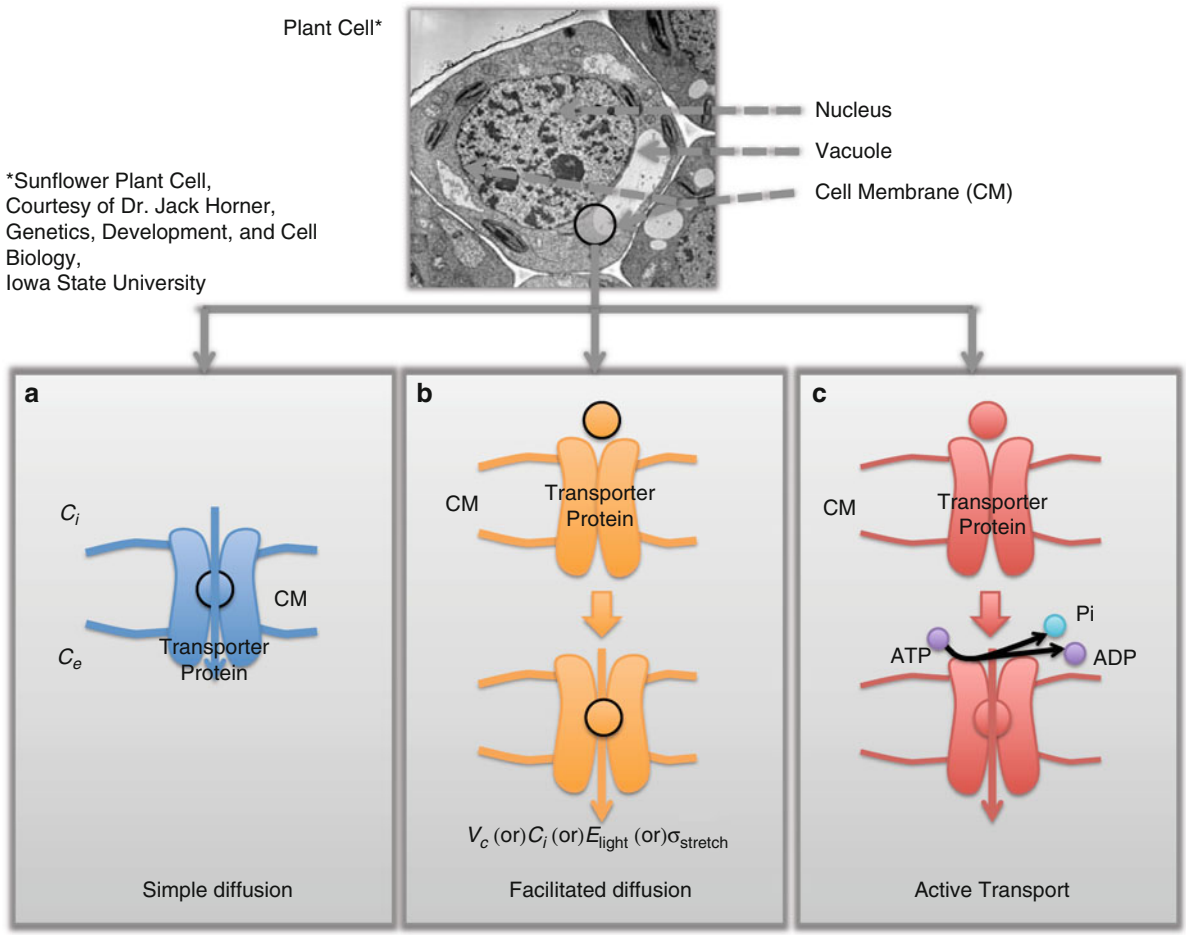
Cell Membrane Proteins: Transporter Macromolecules

The cell membranes of plant and animal cells are host to cholesterol and a variety of proteins that provide structural rigidity and serve as anchor points for cell attachment. In addition, some proteins serve as transporters and allow the cell to exchange ions and neutral molecules between the cell cytoplasm and its surroundings. The transport of species through a protein transporter in the cell membrane is driven by biological processes that have inspired the development of novel sensing, actuation, and energy harvesting concepts. This entry will provide the technical background on biological processes that have inspired the development of *bioderived smart materials* and discuss the system level concepts, fabrication, and characterization of these material systems. Specific emphasis will be given to novel actuation systems, sensing platforms, and recent advances in fabrication of these materials systems.

Transporter proteins are biological macromolecules embedded in cell membranes that convert electrical, mechanical, or chemical stimuli into ion transport and regulate chemoelectrical potentials across the membrane. It is this stimuli response of protein transporters that makes it viable for the development of a bioderived smart material using biological ion transport processes. The transport of ions through the transporter protein occurs via one of the following processes:

1. Simple diffusion
2. Facilitated diffusion
   a. Voltage-gated diffusion
   b. Ligand-gated diffusion
   c. Mechanically gated diffusion
3. Active transport

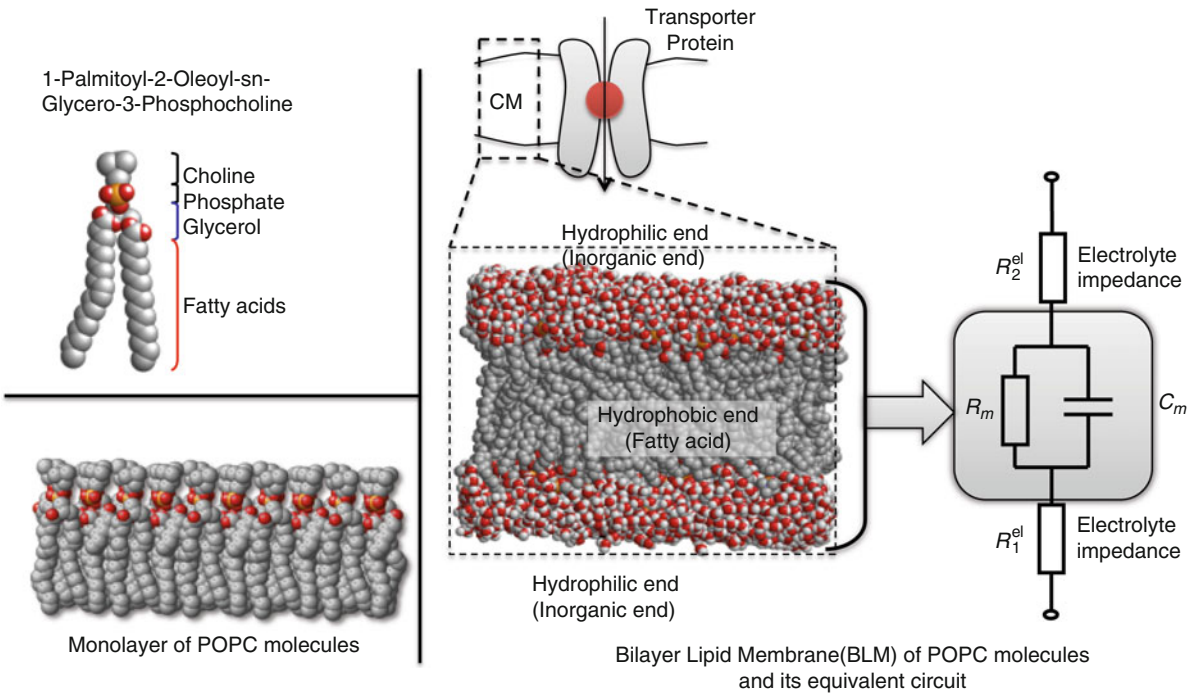
These ion transport processes are illustrated in Fig. 1 and are used to classify protein transporters found in cell membranes. In simple diffusion, the protein transporter behaves like an open channel and allows diffusion of a species along a concentration gradient across the membrane. In facilitated diffusion, the protein transporter uses a gating signal such as light, electrical field, mechanical stretch (or) an analyte to open and conduct an ion or neutral molecule across the membrane. In active transport, the protein transporter uses the energy from biosynthetic molecules such as adenosine triphosphate (ATP) to transport



Bioderived Smart Materials, Fig. 1 Protein transporters in plant cell membrane

ions across the membrane. Since active transporters expend chemical energy available in triphosphates, ions are transported across the membrane against an existing concentration gradient. In addition to ion transport, active transporters establish the necessary chemoelectrical gradients required for facilitated diffusion across the membrane and provide the driving force for long-range ion and solute transport in plant and animal cells.

The mechanism for ion transport through the protein transporter is explained by its molecular composition and structural arrangement. The structural composition of the protein transporter is unique to a plant (or) animal cell membrane and hence transporters are unique in their ion transport function. The diversity of plant and animal cell membranes and the uniqueness of protein transporters found in the cell membranes lead to a large number of protein transporters that can be used as an active component in a bioderived smart material. Protein transporters ion proteins are formed from amino acids that are connected by a polypeptide bond. There are some 20 amino acids that form the majority of the protein transporters and have clearly identified C-C backbone and polar, nonpolar, and aromatic side chains. The linear network of polypeptide bonds has a well-defined two-dimensional structure and is woven (folded) into a three-dimensional structure in the cell membrane. The arrangement of amino acid side chains in the two-dimensional and three-dimensional structures contained within the protein transporters leads to hydrogen bonding, van der Waals, and steric interactions in response to external stimuli. This response in structural changes for an applied optical, electrical,



Bioderived Smart Materials, Fig. 2 l-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine molecule, monolayer, bilayer, and equivalent electrical circuit

mechanical, or chemical stimuli results in the formation of a conductive pathway for a specific ion to migrate across the membrane and results in an ionic current through the protein transporter and the cell membrane carrying the protein transporter. As a consequence of the ionic current, the protein transporter maintains and regulates the electrical potential across the membrane in response to the applied stimulus.

Bilayer Lipid Membranes: A Breadboard for Bioderived Smart Material Concepts

The fabrication of a bioderived smart material requires a membrane that can serve as a host to transporter proteins and exhibit coupling between multiple physical domains (chemical, electrical, mechanical, and optical). This membrane should have the structural properties of a cell membrane and assume the ion transport function imparted by the transporter protein. In addition, this membrane should be sufficiently modular to accommodate multiple proteins and provide a composite ion transport function. The current state of the art in bioderived smart materials uses the same macromolecules that form the cell membranes of plant and animal cells. These molecules, referred to as glycerophospholipids (also referred to as phospholipids or lipids), are extracted and purified from cell membranes and are amphiphilic in nature. Phospholipids comprise of an organic alkyl end group with one or few double bonds and a polar end group connected to each other via a glycerol-phosphate group. An example phospholipid molecule l-*p*almitoyl-2-*o*leoyl-sn-glycero-3-*p*hospho*c*holine (POPC) with an organic palmitoyl- oleoyl- and polar -choline end group is shown in Fig. 2. These molecules, owing to their amphiphilic nature assemble into a bilayer formed from stacked monolayers with the hydrophilic ends of the monolayer facing outside as shown in Fig. 2. This stacked arrangement of hydrophilic-hydrophobic-hydrophilic groups in the bilayer membrane offers high impedance for transport of ions and neutral molecules through the membrane. The bilayer lipid membrane (BLM) measures 6–10 nm in height, spans 10–20/μm^2 area, and serves as the host to various transporter proteins and forms the basic structure of a bioderived smart material. Since various transporter

proteins with different ion transport functions can be incorporated in the BLM to provide a composite ionic function, the BLM becomes the breadboard for various smart material concepts. Owing to their biological origin, the BLM with the protein transporter is referred to in this entry as the bioderived membrane.

In order to quantify the ionic current through the bioderived membrane in the presence of the stimulus, it is required to establish a measure of baseline ion transport (current) through the BLM and charge separation (electrical potential) across the membrane without the protein transporter. The conductance of the membrane is represented using a frequency-dependent complex impedance function and the membrane is modeled using electrical equivalents as shown in Fig. 2. The complex impedance is measured using electrical impedance spectroscopy (EIS) and fitted to an equivalent circuit. In the absence of protein transporters in the BLM, the membrane has a high electrical impedance ($\sim$**O** 0.1–1 GΩ.cm^2) and a capacitance of 1/μF.cm^{-2} [1, 2]. In the presence of protein transporters, the membrane assumes the transport properties of the protein and has markedly different conductance states. The impedance of the membrane with the protein in the presence of the stimuli measured using EIS and the conductance states through cyclic voltammetry (CV) and chronoamperometry (CA) establish the incorporation and functioning of the protein transporter in the membrane.

Concepts for Ionic Active Materials Using Bioderived Membranes

The design of smart materials system using protein transporters employs one or several of the previously discussed ion transport processes. In order to understand the energy conversion processes in a protein transporter and its performance as an ionic active material, it is necessary to quantify the work performed by the bioderived membrane for an applied input energy. The electrical work done by bioderived membranes from ion transport is

$$\Delta U_E = \int_0^q E dQ \tag{1}$$

where E is the electrical field established by the protein and dQ is the charge displacement through the membrane. The electrical field established across the membrane is given by $E = V/t_m$ where V is the transmembrane potential and t_m is the thickness of the membrane. The charge displacement dQ is due to the ionic current i_c through the protein transporter embedded in the membrane and can be obtained from electrochemical measurements on the membrane. The ionic current results in altering the concentration of the ion across the membrane that sets up an additional transmembrane potential V_c give by

$$V_c^{\infty} = \frac{RT}{zF} \ln\left(\frac{C_e^{\infty}}{C_i^{\infty}}\right) \tag{2}$$

where C_e^{∞}, C_i^{∞} are the concentration of the ion on either sides of the membrane at the end of the process and z is the valency on the transported ion. Similarly, the chemical energy gradient across the membrane due to a concentration gradient is given by

$$\Delta\mu = RT \ln\left(\frac{y_e^{\infty}}{y_i^{\infty}}\right) \tag{3}$$

where y_e^{∞}, y_e^{∞} are the mole fractions of species y on either side of the membrane at steady state. The interconversion between chemical potential and electrical potential across the membrane shown in Eqs. 1, 2, and 3 occur via ionic currents through the protein transporter.

The ionic currents through the bioderived membrane can be written for various transport process from *phenomenological equations* as a function of one or more of the following as shown in Sundaresan et al. [3]

1. Applied transmembrane potential(V)
2. Chemical energy gradient($\Delta\mu$)
3. Chemical energy released from biochemical reaction($\Delta\mu$)

The diffusion ionic current through the membrane of area A and permeability constant P_m in the presence of concentration gradients C_e, C_i, and transmembrane potential V is given by Goldman-Hodgkin-Katz equation as

$$i_{\text{diff}} = -AP_mF\left(\frac{zFV}{RT}\right)\left[\frac{[C]_i - [C]_e e^{-\frac{zFV}{RT}}}{1 - e^{-\frac{zFV}{RT}}}\right]. \tag{4}$$

The ionic current through the protein transporter via voltage-gated diffusion i_{vg} is given by

$$i_{vg} = 2\psi zekT\sqrt{[C]_e[C]_i}\frac{A_p}{\in d}\sinh\left(\frac{e(V-Vc)}{2kT}\right), \quad (5)$$

where ψ is the voltage gating coefficient, z is the valency of the transported species, e is the charge on the ion, A_p is the area of the ion conducting channel, and d is the length of the pore in the protein transporter. The ionic current through the protein transporter via active transport i_{pump} is given by

$$i_{pump} = Me\lambda\tanh\left(\frac{F[-V-V_{ATP}+V_c]}{2kT}\right), \quad (6)$$

where M is the pump density in the membrane, z is the charge of the pumped ion, λ is the sum of the forward and backward reaction rates in the pump, V_c is the electrochemical gradient for the pumped ion given by Eq. 2, and k is Boltzmann constant.

The choice of transporter protein in bioderived materials depends on the nature of the stimulus to be detected for sensing, stimulus that is available for actuation and ambient energy source available for harvesting. This choice is also dependent on the ability to extract a protein transporter from a cell membrane and the ability to reconstitute the cell membrane-like structure on a synthetic platform. Thus, a bioderived membrane measuring 6–10 nm in thickness with finite number of transporter proteins supported or suspended on a solid substrate and membrane area of ~10 μm^2 serves as the fundamental unit in a bioderived smart material. In an actuator, sensor, or energy harvesting device, a large number of such membranes with protein transporters are assembled to work in parallel as a smart material. An overview of concepts that use the bioderived membrane as the active component in sensors, actuators, and energy harvesting devices is shown in Fig. 3. The ionic currents through the membrane can be obtained from the ionic current through the transporter from expressions similar to Eqs. 4–6 for the design of smart material systems.

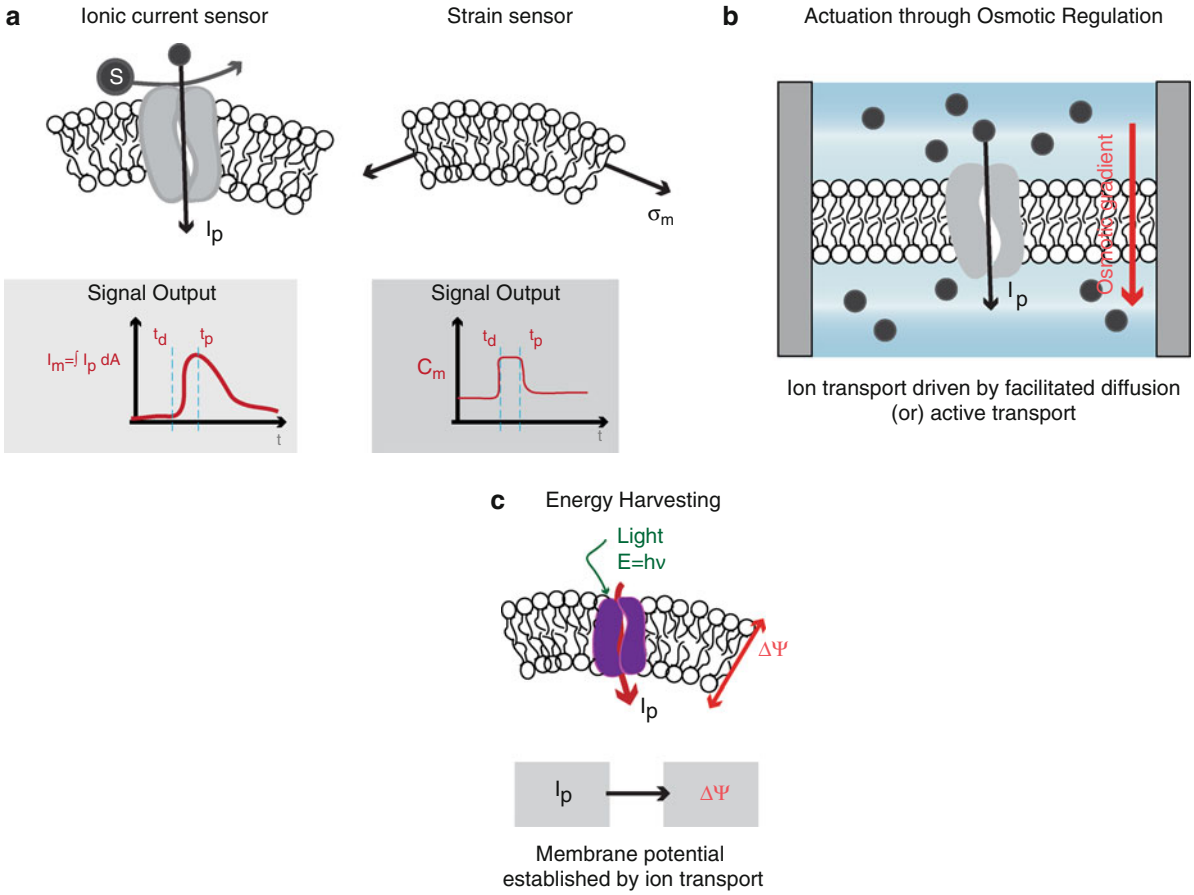
Sensor: The bioderived membrane is used as the sensing element by monitoring the electrical response of the membrane in response to an applied stimulus. The bioderived membrane is formed with electrodes on either sides of the membrane on a synthetic substrate and packaged into a system for monitoring electrical current, transmembrane voltage, or electrical impedance. With the choice of an appropriate protein transporter, the sensor is tailored to respond to chemical analyte, bioelectrical sensors, mechanical strain, light, and temperature.

Actuator: A bioderived membrane is used to regulate the ionic concentration between two closed volumes in response to an applied stimulus. The ionic currents through the bioderived membrane sets up ionic and osmotic gradients across the membrane. This concentration gradient becomes an intermediate stimulus for osmotic regulation and movement of the solvent molecules (water) across the membrane. The bulk movement of water molecules leads to volumetric expansion of a closed volume that will lead to bulk stress in the material. The bulk stress generated in a closed volume encompassing the bioderived membrane is placed in a system that will lead to the development of a stack- or a bimorph-type actuator.

Energy Harvesting: The definition of an energy harvesting device has evolved over the last decade and is now defined as a system that converts ambient energy into electrical energy that can be stored locally and used later for powering electronic devices. In this context, a bioderived membrane generates a chemoelectrical potential from ion transport by varying the ionic concentration as shown in Eq. 2. The concentration gradient and the resulting chemoelectrical potential can be generated by active transport using incident light, consuming triphosphates (adenosine, guanosine), etc. Due to the nature of the ionic transport processes and the resulting gradient across the membrane, protein transporters behave like constant current power sources.

Fabrication Methods and Recent Advances

The methods to form BLM utilize the amphiphilic nature of the phospholipid molecule to produce a highly organized structure in the presence of an aqueous medium. In the past five decades, many techniques have emerged to assemble phospholipids into vesicles and planar BLM [1, 4–8]. Vesicles are hollow-spherical structures that are useful as carrier vehicles for chemical payloads. The planar configuration of the lipid membrane (planar BLM) permits access to both

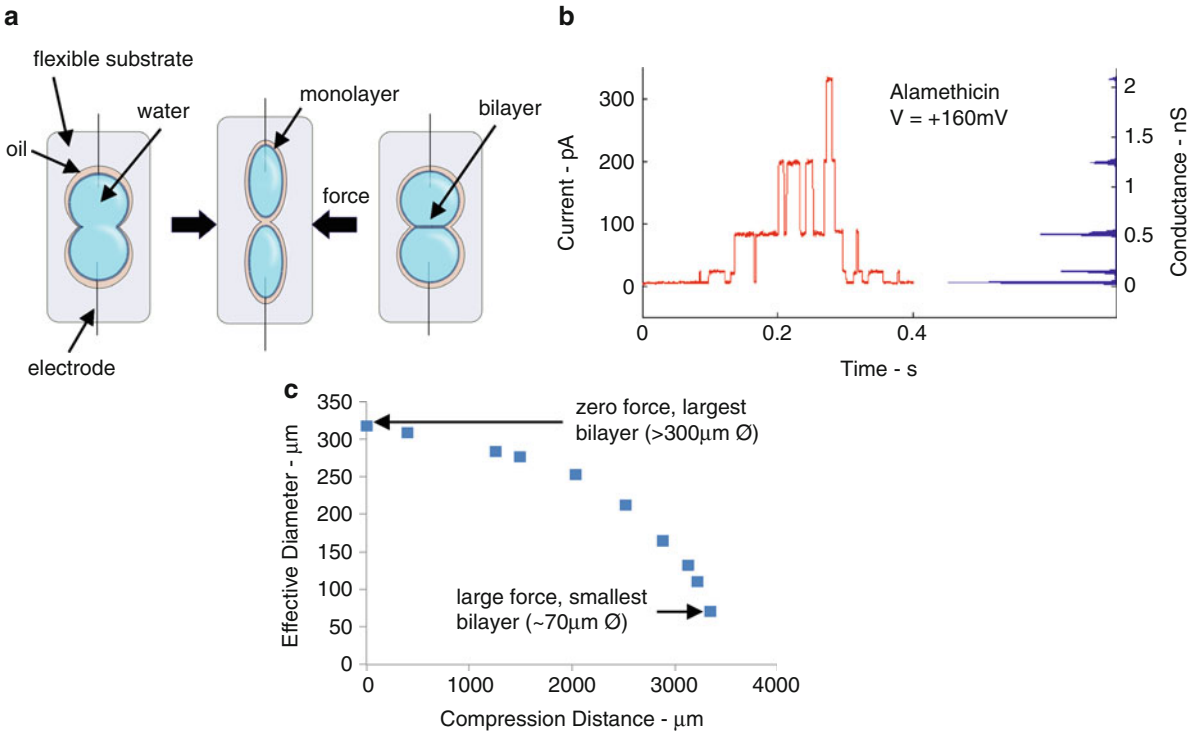


Bioderived Smart Materials, Fig. 3 Concepts for (**a**) sensor elements, (**b**) actuation units, and (**c**) energy harvesting cells using bioderived membranes

sides of the membrane and is preferred for the investigation of ion transport through transporter proteins. The bioderived smart materials typically use a planar BLM supported on a solid substrate (supported BLM) or suspended across the pores of a nanofabricated porous substrate (suspended BLM).

The tools and methods to fabricate a synthetic biological membrane trace its origin to the pioneering research by Mueller and Rudin and coworkers [1, 4] and Tien, Ottova, and coworkers [9]. The initial studies focused on forming planar BLMs from egg phospholipids across a 200-μm pore in a polymeric membrane with voltage-gated ion channels [4]. These initial experimental investigations and subsequent advances by Tien, Ottova, and coworkers were motivated by the need to create in vitro test platforms for analyzing the physiological and immunological function of cell membranes [10–12]. Recently, the experimental methods to form a BLM with proteins are motivated by the engineering applications of bioderived membranes. The anticipated applications for bioderived membranes have significantly influenced the fabrication methods and material choice in the past decade.

A consistent challenge in transitioning supported and suspended lipid bilayers into robust material platforms is the fragility and limited shelf life of the thin membrane. Various approaches have been investigated to stabilize lipid bilayers, including suspending bilayers across nanopores, tethering bilayers to solid surfaces, and sandwiching suspended membranes between water-swollen hydrogels. Among them, this entry will discuss a novel platform for reconstituting phospholipids into a BLM in a curable polymerizable structure. The method, referred to as droplet interface bilayer (DIB), utilizes the affinity of the hydrophobic tails to organic solvents (or) oils. The DIB technique



Bioderived Smart Materials, Fig. 4 (**a**) The regulated attachment method uses a flexible substrate and applied force to enable bilayer formation. (**b**) Membranes produced via the RAM enable single-channel measurements. (**c**) Modulating the applied force allows for reversible control on the size (area) of the bilayer

first demonstrated by Funakoshi et al. [13] and then refined and expanded by Bayley et al. [14–16] showed that the stability of the bilayer can be improved by minimizing the direct interaction of phospholipids with a solid substrate. This leads to a BLM with improved longevity (DIBs lasted for days to weeks) and increased resistance to rupture. In this approach, a liquid-supported interface bilayer is formed by connecting two lipid-encased water droplets submerged in oil as shown in Fig. 4. The monolayers that make up the bilayer self-assemble at the oil-water interface surrounding each droplet and spontaneously "zip" together when the droplets are placed into contact. The formation of a lipid bilayer at this interface occurs by thinning when depletion flocculation removes excess oil from between the droplets. Using this principle, the amount of contact between two droplets can be modulated by the choice of oil.

Aside from advantages of simplicity and stability, the DIB embodiment affords the ability to form a biomolecular network of lipid bilayers by connecting more than two droplets. The ability of the DIB membrane to accommodate proteins makes it feasible to demonstrate selective transport across multiple interfaces formed from a network of droplets. The primary disadvantage of this method, though, is the need to dispense and position individual water droplets a task that becomes increasingly difficult for small droplets (<100 μm). To remedy this limitation and to provide added support to the droplets themselves, Sarles and Leo recently developed an alternative technique called the regulated attachment method (RAM) for interface bilayer formation within a flexible, nonwetting solid substrate [17, 18]. Instead of positioning droplets individually, the RAM uses mechanical force applied to the flexible substrate to control the attachment of lipid-coated aqueous volumes contained in neighboring compartments. The applied force regulates the dimensions of the aperture separating the compartment as shown in Fig. 4a. With the initial design, increasing the force closes the aperture and separates the volumes. Relaxing this force, then, opens the aperture and allows the volumes to come into contact. The results of this method proved

that, like DIBs, interface bilayers formed using RAM lead to stable membranes that exhibit high electrical resistances (>10GΩ) necessary for measuring single-channel and multiple-channel ion currents through proteins such as alamethicin (Fig. 4b). Further, the RAM enables the size of the bilayer (i.e., the area of contact) to be reversibly modulated after membrane thinning. It has been shown through experiments that the effective diameter of the bilayer formed from RAM technique could be varied by a factor of 5X by merely modulating compressive force (Fig. 4c). The flexibility of the regulated attachment method (RAM) provides additional advantages over earlier methods to form lipid bilayers, including the DIB approach. First, this method provides control of the size of the bilayer, independent of the sizes or shapes of the aqueous volumes. Sarles and Leo later utilized this advantage in forming interface bilayers between nonspherical hydrogels [19]. Second, supporting the aqueous volumes within a flexible substrate helps to reduce relative motion between the connected volumes under vibration and shock, making for a more portable system. With this added support, additional contents in the form of discrete aqueous droplets can be added to an existing lipid-coated volume via a microchannel or syringe without causing the membrane to rupture. This capability enables biomolecules (or other species) to be added to the membrane at a designated time after bilayer formation.

Bioderived Microhydraulic Actuators

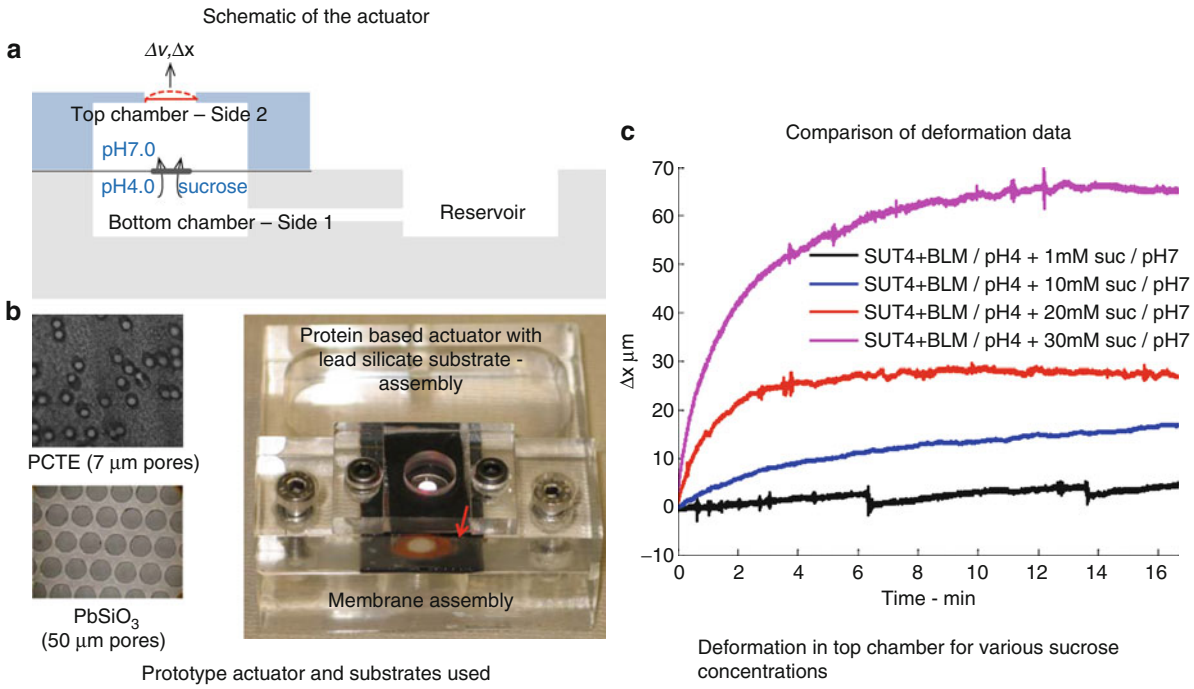
Motion in biomolecules, especially protein transporters, is typically associated with conformational change in proteins and is of the order of few angstroms of displacement and/or angular rotation of the subunits in the protein transporter [20]. There are numerous challenges in coupling this mechanical motion resulting from three-dimensional conformational change of proteins to an external system and hence cannot be used for performing mechanical work. Another interesting example of chemomechanical actuation is the demonstration of kinesin motility on microtubules [21]. In this system, a kinesin-functionalized nanoparticle traverses along the length of a microtubule resulting from the rotation of kinesin dimer from ATP hydrolysis. Various research groups have used nanobeads functionalized with kinesin as platform for demonstrating this novel actuation concept. While this concept is significantly advanced than molecular motors, coupling this linear motion to generate force and volumetric strain has challenges in interfacing the bimolecular motion with a structure. In order to demonstrate a system that uses biomolecules to generate volumetric strain similar to ferroelectrics, electroactive polymers, a membrane-based hydraulic actuation concept was developed by Sundaresan and Leo [3].

The membrane-based microhydraulic actuator uses a BLM with ion transporters as shown in Fig. 5(a–b). The chemomechanical actuator uses osmotic regulation between two chambers resulting from ion and sucrose transport through the bioderived membrane. The bioderived membrane is suspended across the pores of a microporous substrate and separates the contents of the two chambers and is reconstituted with a sucrose transporter protein (SUT4) extracted from *Arabidopsis thaliana*. The SUT4 protein, grown in and extracted from yeast cell membrane, is a cotransporter that transports proton and sucrose from the side of the membrane with higher proton and sucrose concentration. On applying a buffered proton gradient, the cotransporter balances the pH gradient with sucrose concentration gradient. The sucrose concentration gradient generates an osmotic gradient across the membrane and produces water transport through the membrane. The additional volume of fluid from osmotic regulation, demonstrated in Sundaresan et al. [22], builds pressure in the chamber, balances the osmotic gradient established by sucrose transport, and deforms a flexible wall in the chamber.

The mechanics of ion transport leading to an osmotic gradient due to a cotransporter is shown using phenomenological equations. The forces in the system during proton-sucrose cotransport through the membrane for actuation are chemical potentials ($\Delta\mu$) due to

1. Concentration gradient of proton ($\Delta\mu_p$)
2. Concentration gradient of sucrose ($\Delta\mu_s$) – cotransported specie
3. Osmotic gradient due to concentration gradients of charged species and nonelectrolytes

The proton and sucrose fluxes (ϕ_p, ϕ_s) through the membrane due to the applied concentration gradients across a membrane can be represented by phenomenological equations for ion transport formulated by Katachalsky et al. [23, 24].



Bioderived Smart Materials, Fig. 5 (**a**–**b**) Schematic of a membrane-based chemomechanical actuator. (**c**) Deformation of cover plate in the actuator for various initial sucrose concentrations

$$\begin{aligned}\phi_s &= L_{s,s}\,\Delta\mu_s + L_{s,p}\,\Delta\mu_p \\ \phi_p &= L_{p,s}\,\Delta\mu_s + L_{p,p}\Delta\mu_p.\end{aligned} \tag{7}$$

Assuming unity values for activity coefficients, the chemical potentials that contribute to the flux are [25]

$$\begin{aligned}\Delta\mu_p &= RT\ln\left(\frac{c_1^p}{c_2^p}\right) \\ \Delta\mu_s &= RT\ln\left(\frac{c_1^s}{c_2^s}\right).\end{aligned} \tag{8}$$

The coefficients in the flux equations in Eq. 7 represent the coupling between proton and sucrose transport through the membrane. The coefficient $L_{s,s}$ represents the coupling between sucrose chemical potential and sucrose flux. The coefficient $L_{s,p}$ relates sucrose flux to the chemical potential due to proton and similarly, $L_{p,s}$ relates the proton flux to sucrose chemical potential. The coefficients $L_{s,p}$, $L_{p,s}$ are assumed equal due to the Onsager symmetry relationship [26]. The chemical potentials due to sucrose and pH gradients are assumed to balance each other as shown in Sundaresan and Leo [22] and the equilibrium concentration of sucrose is obtained for various pH and sucrose gradients. For an infinite source of sucrose on side 1 of the membrane and zero initial concentration of sucrose on side 2 of the membrane, it is assumed that the concentration on side 1 of the membrane remains unchanged from the initial concentration. The concentration of sucrose on side 2 balanced by a buffered pH gradient at equilibrium is given by the expression

$$c_2^s = c_1^s 10^{\Delta pH}, \tag{9}$$

where $\Delta pH = pH_2 - pH_1$. In the presence of a limited source of sucrose on side 1 of the membrane as in the actuator, sucrose is transported through the membrane until the concentration gradient of sucrose balances the applied proton gradient. The balancing concentration as a function of the volume ratios. The number of moles of the sucrose on side 1 and side 2 at equilibrium is denoted by $n_1^s|_{eq}$ and $n_2^s|_{eq}$, respectively. The volume of the chamber on side 1 and side 2 of the membrane is represented by V_1 and V_2 and the volume ratio by

$V_r = V_1/V_2$. At equilibrium condition the number of moles in the system is constrained by

$$n_1^s|_{eq} + n_2^s|_{eq} = V_1 c_1^s \tag{10}$$

The equilibrium concentration on side 2 of the membrane from Eq. 9 is rewritten in terms of the number of moles of the sucrose on both the sides of the membrane,

$$n_2^s|_{eq} = n_1^s|_{eq} \frac{V_r}{10^{\Delta pH}} \tag{11}$$

The concentration on side 1 at equilibrium condition is obtained in terms of the initial sucrose concentration on side 1 of the membrane by substituting the mass balance expression in Eq. 10 into Eq. 11

$$c_1^s|_{eq} = c_1^s \frac{V_r}{V_r + 10^{\Delta pH}}. \tag{12}$$

Similarly, the concentration of sucrose at equilibrium on side 2 of the membrane is

$$c_2^s|_{eq} = c_1^s \frac{10^{\Delta pH} V_r}{V_r + 10^{\Delta pH}}. \tag{13}$$

The equilibrium concentration of sucrose on side 2 of the membrane $(c_2^s|_{eq})$ due to an imposed initial sucrose concentration (c_1^s) is computed for a volume ratio $V_r = 100$ in Table 1. It is observed from the table that the proton gradient concentrates sucrose on side 2 of the membrane. From the applied proton gradient and different initial concentration of sucrose on side 1 of the membrane, the membrane with cotransporter will develop different osmotic pressures on side 2 of the membrane within a finite duration. This analysis shows the feasibility to use concentration of sucrose as a control variable in the actuator with a cotransporter for modulating the force and deformation. A prototype chemomechanical actuator similar to the schematic in Fig. 5a was fabricated to demonstrate the concept of microhydraulic actuation using bioderived membranes. The porous glass plate is attached to a 500-μm Kapton film and sandwiched in between the two chambers with a rubber gasket. This serves as the supporting substrate for forming suspended BLM with SUT4 transporters. A clamping plate screwed onto the top chamber holds the PET (polyethylene terepthalate) coverplate that deforms out of the chamber due to increasing pressure from osmotic regulation across the two chambers. The volumes of the chambers are designed to be 0.54 ml and 50 ml so that the larger chamber resembles a reservoir. The procedure to assemble the actuator follows the description in Sundaresan and Leo [22]. The BLM is formed from POPS and POPE lipids by painting method and the proteins are reconstituted by vesicle fusion method. In this method, 10 μl of lipids (POPS:POPE mixed in 3:1 w/w ratio and dissolved in n-decane at 40 mg/ml) is painted on the surface and allowed to dry for 10 min under a stream of nitrogen. This is followed by the addition of 10 μl of SUT4 transporters in liposomes suspended in pH7.0 medium. The porous substrate with added components are allowed to stand in air for 15 min and assembled into the actuator.

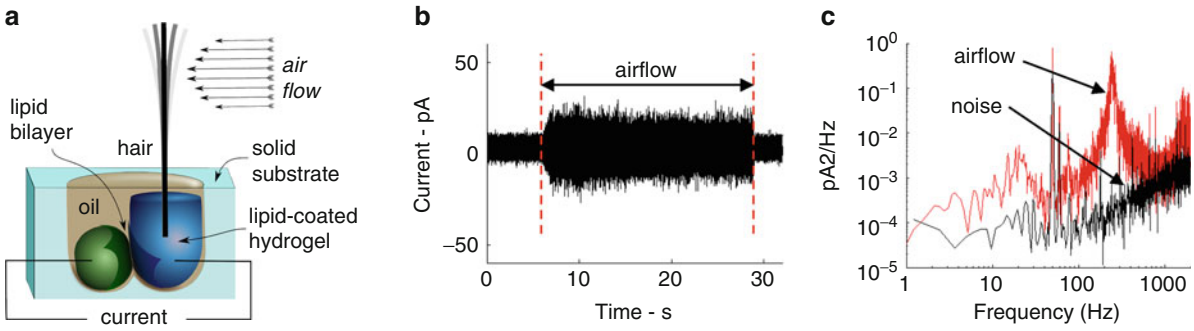
Bioderived Smart Materials, Table 1 Equilibrium concentration of sucrose on side 2 of the membrane in the actuator for pH4/pH7 applied across the membrane assembly

c_1^s mM (Initial)	1	10	15	20
$c_2^s|_{eq}$mM	90	900	1,360	1,810

The assembled prototype actuator is characterized by applying different concentrations of sucrose in the bottom chamber and measuring the deformation of the coverplate. As a control study, different baseline tests with a key ingredient left out is tested. The different baseline tests performed on the actuator are:

1. BLM with pH7.0 buffer on both sides of the membrane
2. BLM with pH4.0/pH7.0 gradient without sucrose dissolved in the bottom chamber
3. BLM with pH4.0/pH7.0 gradient with 5 mM sucrose dissolved in the bottom chamber

The results from baseline tests on the prototype shown in Sundaresan and Leo [22] demonstrate that there is no deformation in the coverplate for the case with pH7.0 buffer on both the sides of the membrane. Similarly, the coverplate does not show an appreciable deformation for a pH gradient applied to the membrane without sucrose. In the third baseline, pH gradient is applied across a BLM without SUT4 and 5 mM sucrose is added to the bottom chamber. It is observed that the coverplate deforms into the top chamber and is attributed to the migration of water molecules from the top chamber to the bottom chamber driven by the osmotic gradient.



Bioderived Smart Materials, Fig. 6 A membrane-based hair cell (**a**) uses the mechanoelectrical properties of a lipid bilayer to transduce airflow into current (**b**). (**c**) The frequency content of the measured signal correlates to the mechanical vibrations of the hair

The next set of experiments are performed on an actuator assembled with pH4.0/pH7.0 gradient applied across the BLM with SUT4 and different concentrations of sucrose. Representative results from these experiments are shown in Fig. 5c. From the experimental results in Fig. 5c, it is observed that the deformation in the actuator and rate of deformation increases with the initial sucrose concentration on side 1 of the membrane. The performance metrics of this actuator of this bioderived membrane-based actuator discussed in Sundaresan and Leo [22] compares well with soft polymeric electrochemomechanical actuators [27]. Thus, this demonstration follows the theoretical discussion that varying the sucrose concentration across the SUT4 cotransporter results in an osmotic gradient and leads to volumetric strain in the flexible wall of the actuator.

Hair Cell Sensors

Sensory hairs, or hair cells, are one of the most common forms of transducers found in nature. In general, a hair cell is type of sensory receptor that uses protruding structures, called cilia, to probe the surrounding environment. Sharing this basic trait, natural hair cells display tremendous diversity in their morphologies, mechanical properties, and physiological functions. As a result, animals use hair cells to detect sound, pressure, flow, vibration, chemical species, and even position (inertial tilt). Motivated by the diversity of stimuli-responsive behavior of natural hair cells, several groups have used synthetic active materials to engineer hair cell-inspired sensors. Most notably, Liu's research group [28] pioneered multiple generations of artificial cilia that used microfabricated cilia mounted on strain gauges or polymeric force-sensitive resistors (FSRs) to create an electrical response in response to flow or touch. However, living hair cells such as mammalian outer hair cells (OHCs) rely heavily on soft biological materials, namely, cell membranes and stretch-activated ion channels to sense deflections the cilia.

Building on recent advances to assemble and encapsulate an artificial cell membrane within a flexible substrate, Sarles et al. [29] demonstrated that a gel-supported lipid bilayer could be used to build a new type of membrane-based hair cell-inspired sensor. As shown in Fig. 6a, the hair cell consists of synthetic cilium (hair) that features an electrolyte-swollen polymeric gel at lower end and which is held vertically within one compartment of a nonswelling solid substrate. A second, lipid-encased aqueous volume contained in the neighboring compartment connects to the gel at the base of the hair to form a lipid bilayer. Both compartments also contain immiscible oil needed for promoting phospholipid self-assembly at the oil-water interfaces. The performance of the hair cell sensor was characterized by measuring the current through the membrane (using conductive probes inserted into each aqueous volume) in response to the applied airflow across the hair. The results of this initial study demonstrated that the membrane-based hair cell generates an increase in the amplitude of the current through the bilayer in response to flow-induced vibration of the synthetic hair (Fig. 6b). Also, the power spectral density revealed that the frequency content of the sensing current (Fig. 6c) differs from that of the background noise evidence that the mechanical vibrations of the hair contribute to the measured

current. Unlike living hair cells that use stretch-activated ion channels to provide both static and oscillatory sensing, this system only contained an Ac response (i.e., static deflection of the hair did not result in a change in the current). Yet, these initial responses did not explain the source of the current. By systematically varying the applied potential across the bilayer and holding the area of the membrane constant during the experiment, the authors discovered that the sensing current increased linearly with respect to the voltage. This result provided proof that a change in the electrical capacitance of the membrane, caused by bending in the bilayer, is the source of the measured current. In this work, the sensing currents ranged from 10 to 100 pA, depending on the speed of airflow, the length of the hair, and the transmembrane potential. Current efforts to increase the sensitivity of the sensor, provide static and directional responses, and fully encapsulate the liquid contents are underway.

Concluding Remarks

This entry presents a novel framework to develop smart materials using biomolecular components extracted from cell membranes. The bioderived smart materials use ion and fluid transport through protein transporters embedded in an impervious membrane and couple one or more of the following – chemical, electrochemical gradients across the membrane, mechanical stretch in the membrane and light energy incident on the membrane. This entry discusses a unique bioderived actuator that uses sucrose transport through a proton-sucrose transporter to generate volumetric strain. It is shown that the blocked force generated by these materials resemble soft polymeric electrochemomechanical actuators. We also present a novel electromechanical sensing concept using the BLM and demonstrate the potential of this device as a mechanical flow sensor. In the bioderived smart material systems described in this entry, two methods to fabricate the bioderived membrane are presented. Among the two methods, the first method utilizes the self-assembly of phospholipids in a pore to form a BLM with proteins. The second method, inspired by DIB, demonstrates an innovative method to fabricate a durable bilayer membrane, demonstrates the force and flow sensing, and provides a rugged framework for future development in this field. The framework for building bioderived smart materials and their application as sensors, chemomechanical actuators, and energy harvesting devices discussed in this entry benefits from a wide variety of transporters that can be extracted from cell membranes and offers innovative approaches to couple various forms of energy.

Cross-References

- ▶ Biomimetic Flow Sensors
- ▶ Biomimetics
- ▶ Biosensors
- ▶ Molecular Dynamics Simulations of Nano-Bio Materials
- ▶ Organic Actuators
- ▶ Organic Bioelectronics
- ▶ Synthetic Biology

References

1. Montal, M., Mueller, P.: Formation of bimolecular membranes from lipid monolayers and a study of their electrical properties. Proc. Nat. Acad. Sci. USA **69**(12), 3561–3566 (1972)
2. Ottova, A.L., Ti Tien, H.: Self-assembled bilayer lipid membranes: from mimicking biomembranes to practical applications. Bioelectrochem. Bioenerg. **42**(2), 141–152 (1997)
3. Sundaresan, V.B., Homison, C., Weiland, L.M., Leo, D.J.: Biological transport processes for micro-hydraulic actuation. Sens. Actuators B Chem. **123**, 685–695 (2007)
4. Mueller, P., Rudin, D.O., Tien, H.T., Wescott, W.C.: Reconstitution of excitable cell membrane structure in vitro. Circulation **26**(5), 1167–1171 (1962)
5. Needham, D., Haydon, D.A.: Tensions and free energies of formation of "solventless" lipid bilayers measurement of high contact angles. Biophys. J. **41**(3), 251–257 (1983)
6. Poulin, P., Bibette, J.: Adhesion of water droplets in organic solvent. Langmuir **14**(22), 6341–6343 (1998)
7. Haydon, D.: Properties of lipid bilayers at a water-water interface. J. Am. Oil Chem. Soc. **45**(4), 230–240 (1968)
8. Fettiplace, R., Andrews, D.M., Haydon, D.A.: The thickness, composition and structure of some lipid bilayers and natural membranes. J. Membr. Biol. **5**(3), 277–296 (1971)
9. Tien, H.T., Wurster, S.H., Ottova, A.L.: Electrochemistry of supported bilayer lipid membranes: background and techniques for biosensor development. Bioelectrochem. Bioenerg. **42**, 77–94 (1997)
10. Tien, H.T.: Self-assembled lipid bilayers as a smart material for nanotechnology. Mater. Sci. Eng. C. **3**, 7–12 (1995)

11. White, S.H.: Analysis of the torus surrounding planar lipid bilayer membranes. Biophys. J. **12**(4), 432–445 (1972)
12. Singer, S.J., Nicolson, G.L.: The fluid mosaic model of the structure of cell membranes. Science **175**(4023), 720–731 (1972)
13. Funakoshi, K., Suzuki, H., Takeuchi, S.: Lipid bilayer formation by contacting monolayers in a microfluidic device for membrane protein analysis. Anal. Chem. **78**(24), 8169–8174 (2006)
14. Holden, M.A., Needham, D., Bayley, H.: Functional bionetworks from nanoliter water droplets. J. Am. Chem. Soc. **129**(27), 8650–8655 (2007)
15. Hwang, W.L., Holden, M.A., White, S., Bayley, H.: Electrical behavior of droplet interface bilayer networks: Experimental analysis and modeling. J. Am. Chem. Soc. **129**(38), 11854–11864 (2007)
16. Bayley, H., Cronin, B., Heron, A., Holden, M.A., Hwang, W.L., Syeda, R., Thompson, J., Wallace, M.: Droplet interface bilayers. Mol. Biosyst. **4**(12), 1191–1208 (2008)
17. Sarles, S.A., Leo, D.J.: Regulated attachment method for reconstituting lipid bilayers of prescribed size within flexible substrates. Anal. Chem. **82**(3), 959–966 (2010)
18. Sarles, S.A., Leo, D.J.: Membrane-based biomolecular smart materials. Smart Mater. Struct. **20**(9), 094018 (2011)
19. Sarles, S.A., Stiltner, L.J., Williams, C.B., Leo, D.J.: Bilayer formation between lipid-encased hydrogels contained in solid substrates. ACS Appl. Mater. Interfaces **2**(12), 3654–3663 (2010)
20. Oster, G., Wang, H.: Rotary protein motors. Trends Cell Biol. **13**(3), 114–121 (2003)
21. Hess, H., Bachand, G.D., Vogel, V.: Powering nanodevices with biomolecular motors. Chem. A Eur. J. **10**(9), 2110–2116 (2004)
22. Sundaresan, V.B., Leo, D.J.: Modeling and characterization of a chemomechanical actuator using protein transporters. Sens. Actuators B Chem. **131**(2), 384–393 (2008)
23. Kedem, O., Katchalsky, A.: A physical interpretation of the phenomenological coefficients of membrane permeability. J. Gen. Physiol. **45**(1), 143–179 (1961). doi:10.1085/jgp.45.1.143. arXiv: http://www.jgp.org/cgi/reprint/45/1/143.pdf
24. Schultz, S.G., Curran, P.F.: Coupled transport of sodium and organic solutes. Physiol. Rev. **50**(4), 637–718 (1970). arXiv: http://physrev.physiology.org/cgi/reprint/50/4/637.pdf
25. Gyftopoulos, E.P., Berreta, G.P.: Thermodynamics: foundations and applications. Macmillan, New York (1996)
26. Onsager, L.: Reciprocal relations in irreversible processes. II. Phys. Rev. **38**(12), 2265–2279 (1931). doi:10.1103/PhysRev.38.2265
27. Akle, B., Bennett, M., Leo, D.J.: Development of a novel electrochemically active membrane and "smart" material based vibration sensor/damper. Sens. Actuators A Phys. **126**(1), 173–181 (2006)
28. Liu, C.: Micromachined biomimetic artificial haircell sensors. Bioinspir. Biomim. **2**(4), S162 (2007)
29. Sarles, S.A., Madden, J.D.W., Leo, D.J.: Hair cell inspired mechanotransduction with a gel-supported, artificial lipid membrane. Soft Matter **7**(10), 4644–4653 (2011)

Bio-FET

► Nanostructure Field Effect Transistor Biosensors

Biofilms in Microfluidic Devices

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Synonyms

Floccules; Microbial aggregations

Definition

Biofilms are aggregations of microbes that are encased by extracellular polymeric substances (EPS) and adhere to surfaces or interfaces. Biofilms exist in a very wide diversity of environments, and microfluidic devices are being increasingly utilized to study and understand their formation and properties.

Overview

Microbes often form aggregates on interfaces, and due to a production of EPS, the aggregates become encased in a matrix [1]. Though microbes in a biofilm are physiologically distinct from bacteria growing in a free swimming state (planktonic bacteria), biofilm growth is a complex process that is typically initiated by planktonic bacteria themselves. Biofilm growth is initiated with bacterial adhesion to a surface, followed by events such as growth, EPS secretion, and morphological and physiological changes. Microbial biofilms