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Artificial signal transduction across membranes

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Abstract: A key conundrum in the construction of an artificial cell is to simultaneously maintain a robust physical barrier to the external environment, while also providing efficient exchange of information across this barrier. Biomimicry provides a number of avenues by which such requirements might be met. Here we provide a brief introduction to the challenges facing this field and explore progress to date.

Keywords: Artificial Cell • Biomimicry • Nanotechnology • Ion channels • Receptors

Introduction

From an engineering perspective, a cell might be considered an advanced microreactor, capable of processing signals from its environment, converting them into complex responses. Compared to conventional 'hard' devices cells also exhibit unique features that would be useful in order to exploit to create new biologically-inspired technologies based on artificial cells. Recent advances in synthetic biology have put the ambitious goal of constructing an artificial cell within the realms of possibility. Here we focus on a key aspect of this grand challenge, the communication of information across cell membranes.

One difficulty in assessing progress in this area is the diverse and overlapping potential applications for such technology. Such outcomes encompass understanding prebiotic life, building model systems to dissect the rules governing cell biology, developing new methods for biological manufacturing, creating new smart therapeutics, and biological computers.[1–5] Researchers working towards one goal might have very different constraints on what components would be considered appropriate for an artificial cell. Within this broad remit, research can also be classified as 'top-down' or 'bottom-up'. Top-down synthetic biology seeks to adapt and pare-down existing cellular life to create minimal cells and devices optimised for a specific technological outcome. Bottom-up synthetic biology seeks to create artificial cells and devices starting from molecular components. In the context of designing systems for artificial signal transduction we will focus primarily on bottom-up approaches. For an artificial cell to be considered living it must also fulfil a number of basic criteria essential to all living things. Although there is still some variation in this classification, we would follow Gánti's chemoton model, and highlight: (1) A unit cell (with a defining boundary); (2) Metabolism; (3) Self-maintenance; (4) Information storage; (5) Adaptation to its environment.[6,7]

Fundamentally, to interact with its surroundings a cell must transduce signals into and out of the environment. Compartmentalisation is required to separate a cell from its surroundings, typically but not exclusively in the form of a lipid membrane. However, this compartmentalisation also presents a problem: inherent in its function is the prevention of interaction with the surrounding environment - a requirement that is in conflict with the need for such a cell to be able to process information, uptake nutrients, and respond appropriately to changes in the environment to maximise their survival.

Biology has already generated solutions to this problem; adopting similar strategies in an artificial cell might range from the wholescale incorporation of transmembrane proteins or signalling networks, to engineering fully synthetic molecules that seek to replicate the strategies that nature has developed.

Here we seek to provide an overview of the advances in artificial signal transduction across membranes as they might be used in the fabrication of an artificial cell, and how they relate to or differ from their biological counterparts (Fig. 1) – encompassing (1) ion channels; (2) receptors; (3) carriers and pumps; and (4) engaging physical properties of the membrane itself to convey signals.

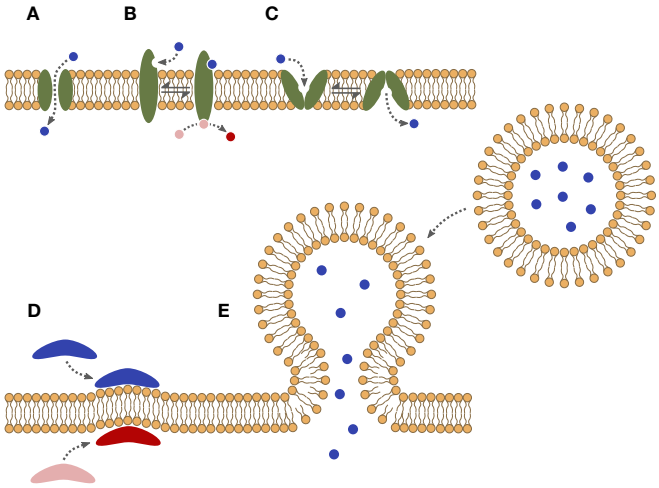

Figure 1. Schematic overview of signal transduction mechanisms: (A) Channels and pores; (B) Receptors; (C) Transporters; (D) Membrane binding; (E) Vesicle fusion.

Ion channels

Lipid bilayers are essentially impermeable to small inorganic ions due to the hydrophobic nature of the bilayer core. To permit exchange of ions across the bilayer, nature has evolved specialized ion channels that act to stabilize hydrophilic ions as they cross the membrane, and proteins that pierce the membrane to form holes. Ion channels transport ions passively down a concentration or electrochemical gradient, with rates of up to 108 ions per second.[8,9] Transport is regulated by controlling the opening and closing of channels (gating) in response to diverse stimuli, including changes in membrane potential, ligand binding, or mechanical force.[10–17] Furthermore, the structural asymmetry observed in rectifying ion channels allow a favored direction for the ion flow. Such channels play a key role in maintaining the membrane resting potential.[18,19] When constructing an artificial ion channel, two obvious requirements must be met: the channel should (1) span the membrane; and (2) mediate a passive ion flux.

Early model ion channels such as gramicidin, alamethicin, and cyclic ion carriers such as valinomycin, and the polyene antibiotic amphotericin B,[20–23] have served as longstanding inspiration when constructing artificial ion channels. The earliest attempts reported used tetra-substituted amphiphilic β-cyclodextrin macrocycles and a membrane-spanning β-helix.[24,25] Subsequently, a variety of synthetic methods have been applied to create new channels; ranging from synthetic macromolecular (in)organic chemistry that uses structural

motifs not appearing in nature, to (artificial) peptide and protein engineering, and DNA nanotechnology.

Synthetic ion channels

Since the 1980s inorganic and organic frameworks provide the mainstay of efforts towards the creation of artificial ion channels that are capable of modulating their response to an external input. Such constructs have been the subject of significant and recent reviews.[26–29] In general, these structures exploit host-guest interactions in stacked assemblies of synthetic frameworks along a membrane-spanning scaffold to provide a conductive pathway for the transport of ions.

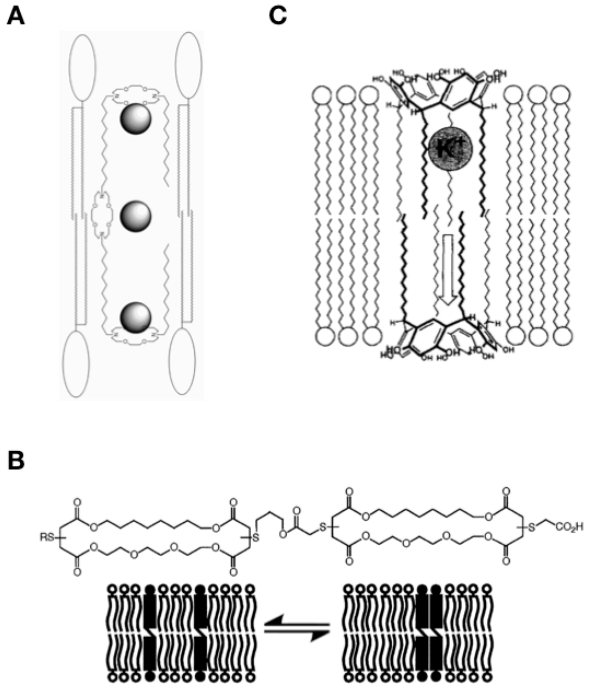

Figure 2. Synthetic ion channels. (A) General structure of Gokel's hydraphiles. Two distal crown ethers interact with the aqueous environment, while a third provides a hydrophilic element in the middle of the bilayer to facillitate ion transport.[9] (B) A representative example of double hydrophilic macrocycles that assemble into ion channels in a lipid bilayer.[35] (C) Resorcin[4]arene selectively transports K+ over Na+, and displays voltage-gating.[40] Images reproduced with permission.

Anion, cation, and salt co-transport was realized in the 1990s and early 2000s by several groups using crown ethers, calix[4]pyrroles, calix- and pillararenes, and steroid derivatives such as cholapods, which are tuneable bile acids that display strong Cl- affinities.[30,31] Notable examples include the tubular structures of Gokel's hydraphiles, consisting of three crown ethers linked via aliphatic chains[9,32,33] (Fig. 2A), and bolaamphiphile designs by Fyles (Fig. 2B), who reported conductances between 10 and 30 picosiemens (pS).[34–36] More recently larger, 800 pS conductances were reported for self-assembled crown ether-based amphiphiles.[37] Crown ethers have also been arranged along other scaffolds, such as peptide-based □-helices[38], and β-barrel-forming octiphenyl rigid rods.[39] Macrocycles,

primarily based on arene- and pyrrole-units, were also implemented in the construction of artificial ion channels. For example, a resorcin[4]arene hemi-channel (Fig. 2C) was the first to report voltage-dependency, with a conductance of ~6 pS.[40] Another example are the ion channels built from the tetrameric macrocycle pyrogallol[4]arene, with conductances between 10 and 460 pS[41–43], and the demonstration of chiral-selective transport of amino acids through peptide-modified pillar[5 or 6]arenes.[44] Lastly, an artificial hemi-channel built from a modified cyclodextrin oligosaccharide macrocycle was reported to selectively transport anions across lipid bilayers, and could also discriminate between halides.[45] More recently, other frameworks such as π-stacked planar architectures and metal organic frameworks have emerged, the former using stacked guanosine tetramers to transport Na+ across a bilayer.[46] Subsequently, this approach was expanded to folate dendrimers[47], stacked macrocycles that assembled into nanotubes with a 5.8 pS conductance[48], and rigid-rod π-slides made from oligo-p-phenylene-N,N-naphthalenediimide that enable transmembrane Cl- transport.[49] Copper-based metal organic cuboctahedrons also reportedly permit selective Li+ transport[50], whereas Tecilia et al. incorporated rhenium in a tetraporphyrin network to construct a non-selective ion channel.[51] Artificial ion channels made from ethylenediamine palladium(II), have been proposed to form long-lived toroidal pores with a conductance of 290 pS.[52] Most recently, efforts have focused on artificial ion channels responsive to stimuli.[53] For example, mimicking of multi-pass transmembrane moieties from oligo(ethylene glycol) chains and aromatic units enabled the construction of an artificial mechanosensitive ion channel (Fig. 3A).[54]

Peptides and peptidomimetics

Lipid bilayers are typically 3 to 4 nm thick but reliably synthesizing systems that can span such distances is not always straightforward.[26] Arguably the simplest natural structures capable of spanning the bilayer come from peptides. In particular, antimicrobial peptides exploit a number of pore-forming mechanisms from which inspiration can be drawn.[55] Examples are the discrete peptide pores formed by the □-helical transmembrane peptide alamethicin[56,57], and the stabilization of toroidal lipid pores by magainin-II.[58,59] Taking this strategy to its extreme, “minimalist” channel forming peptides can be synthesised from heptads of just two amino acids that self-assemble into □-helical bundles in the bilayer.[60,61] These cation-selective channels displayed rectification and channel gating, and although no channel conductivity values were reported, the data presented indicates maximum values around 150 pS.

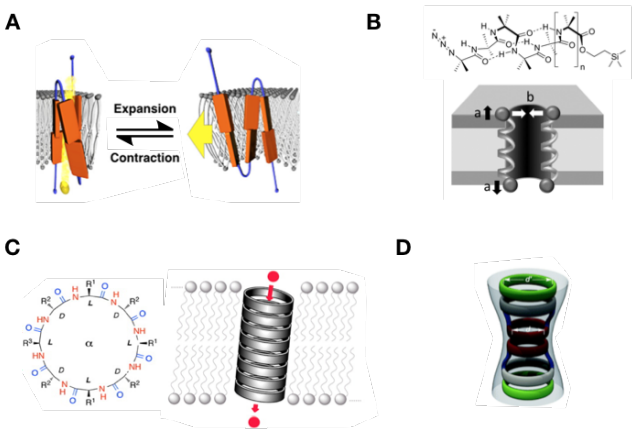

Figure 3. Peptide-based channels. (A) Repeating oligo(ethylene glycol) and aromatic moieties produce multi-pass transmembrane structures that respond to membrane tension. Increasing membrane tension reduced the stacking of the aromatic moieties, reducing ion transport.[54] (B) General structure of α-aminoisobutyric acid (Aib) foldamers, which form helices in membranes that enable ion transport. Largest conductances were found for foldamers consisting of more than 10 Aib units.[65] (C) Rings of cyclic peptides self-assemble into peptide nanotubes in a lipid bilayer, creating a passage for ions.[69] (D) Covalent linking of cyclic peptides with different internal diameters allow the construction of a venturi-like peptide nanotube that allows internal filtering via size-exclusion.[70] Images reproduced with permission.

The same design principle was later extended to include artificial amino acids, which were shown to also adopt helical conformations.[62–64] For example, 310-helical foldamers, consisting of α-aminoisobutyric acid (Fig. 3B) showed channel activity with a conductance of approximately 1500 pS.[65] Alternatively, Yang et al. demonstrated that acyclic small molecules featuring two α-aminoxy acids surrounding an isophtalamide scaffold are excellent anion receptors[66] and potent Cl- transporters, demonstrating two conductance states of 54 and 108 pS.[67]

β-helical peptide structures are also exploited in antimicrobial agents. Gramicidin A is an archetypical example of such a pore forming peptide, where stacked rings of alternating L- and D- α-amino acids form a membrane-spanning pore.[20,68] This strategy was used in the formation of peptide nanotubes (Fig. 3C&D) from self-assembled stacks of cyclic peptides.[69,70] In these structures, the peptide backbone lines the inner hydrophilic core of the pore and the hydrophobic side chains face the lipid bilayer core, based on the flat ring structures adopted by alternating D- and L- α-amino acid sequences.[71,72] Single-channel recordings showed gating behaviour with a conductance of 65 pS. Since then, peptide nanotubes have been studied extensively showing, for example, that larger macrocycles result in larger channels.[73] α,γ-amino acids improve on these designs by providing control over channel properties via functionalization.[74–76] Very recently, stacks of cyclic peptides with different internal diameters were covalently linked to produce a venturi-like nanotube with an internal filter[77], and peptide nanotubes with large internal diameters that could encapsulate C60 fullerene (a “buckyball”) were made by incorporating α,δ-amino acids.[78]

Although no encapsulation in a membrane has been reported for these last two structures, the authors reported ongoing efforts to achieve this.

Protein engineering

Protein engineering encompasses many different techniques to alter the structure of existing proteins or to build de novo structures. One key example of this approach in terms of artificial ion channels is the creation of chimeric fusions of two transmembrane proteins to introduce new functionality. A key example here are the ion-channel-coupled-receptors (ICCRs), where G-protein-coupled receptors (GPCRs) are fused to an existing ion channel.[79] This strategy originates from biology, where, for example, in ATP-sensitive potassium channels (KATP) two subunits are functionally coupled: an inward-rectifying K+ channel, Kir6.2 or Kir6.1, and the sulfonylurea receptor (SUR), which serves as a regulator to tune Kir6.x gating upon ligand-binding.[80] Moreau et al. replaced SUR with various GPCRs, such as the muscarinic M2 receptor and the dopaminergic D2 receptor, to produce a chimeric channel responsive to ligand-binding of acetylcholine and dopamine, respectively.[81] Subsequently, the same group expanded this approach to successful coupling of Kir6.2 to the □2-adrenergic receptor (Fig. 4A)[82], an important drug target because of its involvement in smooth muscle relaxation[83], and to opsin, the GPCR that is part of the light-sensitive rhodopsin[84], to produce a light-sensitive ion channel.[85] Lastly, in 2015, Oh et al. produced an ion channel that responded to odorant simulation by coupling Kir6.2 to human olfactory receptors (hORs).[86]

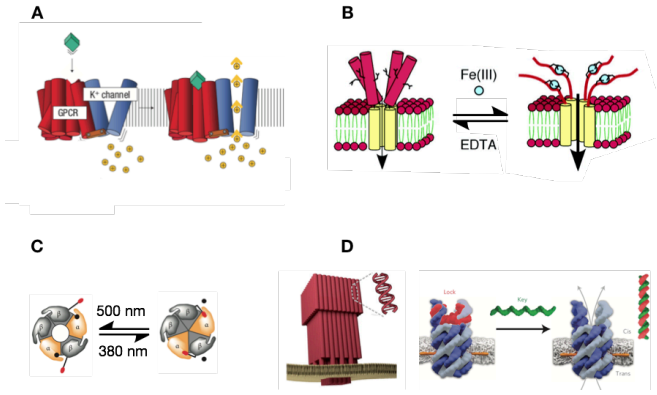

Figure 4. Protein engineering & nanopores. (A) Ion-channel-coupled-receptors fuse G-protein-coupled receptors to existing ion channels.[82] (B) Fusing alamethicin to a leucine zipper creates a metal-gated ion channel. Coordination of iron(III) to the leucine zipper segments opens the ion channel, reversed by EDTA.[92] (C) Ligands tethered to acetylcholine receptors switch conformation upon irradiation with light, and competition of the ligand for acetylcholine binding alters channel gating.[101] (D) Advances in DNA nanotechnology allowed the creation of large pores, e.g. mimicking the size of the nuclear pore complex (left)[135], and ligand-gated ion channels, where a locking strand can be removed via toehold-mediated strand displacement (right).[140] Images reproduced with permission.

An alternative strategy modifies gramicidin A and alamethicin moieties to produce artificial ligand-gated ion channels. Woolley et al. reported the construction of a calcium-responsive

channel by functionalising alamethicin with pyromellitate.[87] Alternatively, streptavidin-biotin has been used to modify gramicidin and alamethicin channel behaviour upon ligand binding.[88–90] Lastly, Futaki and co-workers fused alamethicin to a leucine zipper segment from the cFos protein, creating a metal-gated ion channel (Fig. 4B).[91,92]

Another interesting protein-engineering approach is to functionalize an existing protein structure with photoisomerizable chemical moieties to allow conformational switching between active and inactive states upon irradiation.[93–97] This was first demonstrated by Stankovic et al., who linked two gramicidin A (gA) half-channels together by an azobenzene linker.[98] Upon irradiation, the azobenzene reversibly switches conformation forcing the two gA half-channels to change from an open to a closed state. Other notable examples include modification of MscL from E. Coli to create a light-actuated nanovalve[99], the construction of a photo-switchable SecYEG complex[100], and the control over modified neuronal acetylcholine receptors by combining them with light-sensitive ligands (Fig. 4C).[101]

Nanopores

Arguably the simplest form of ion channel, pore-forming proteins (or nanopores) create large, non-gating, passive channels without intrinsic conformational states.[58] This simplicity is an advantage when considering components from which to build an artificial cell. Pore-forming proteins are commonly found as bacterial toxins such as □-hemolysin,[102] aerolysin[103], or anthrax[104,105]. □-barrel pores are also found as the primary communicating element of the gram-negative bacterial outer membrane.[106,107]

Beyond their biological role, pore-forming proteins have also been shown to act as excellent sensors by measuring the residual ionic flux during the translocation of analytes through the pore.[108] This has been applied to achieve nanopore nucleotide sequencing, where the nucleobase sequence can be measured from an individual translocating nucleic acid polymer.[109,110]

Nanopores can also be used to introduce connectivity between aqueous compartments in networks connected by droplet interface bilayers.[111] Examples are artificial batteries[112], and electronic circuits and components.[113,114] Expanding the scope of these droplet-networks beyond the oil-phase into the aqueous phase allowed the creation of tissue-like material, where pathways can be programmed into by incorporating nanopores in specific droplets.[115–117] Incorporating light-activated □-hemolysin expression allowed the development of functional artificial neuronal transmission.[118]

Recently, porins made from single-wall carbon nanotubes have been developed to mimic protein nanopores.[119] They were shown to spontaneously insert into lipid bilayers, where they display stochastic gating behaviour and a conductance of 70-100 pS.[120] They were shown to diffuse in lipid membranes with diffusion coefficients close their natural counterparts[121], and can transport water, ions, and uncharged species selectively.[122,123]

DNA nanotechnology

DNA nanotechnology[124], and specifically DNA origami, has enabled the construction of complex 3D nanostructures from which artificial channels and pores might be constructed.[125–133] DNA origami uses a long, single "scaffold" strand that is folded into the desired shape by binding to many short "staple" strands. Tight control over the architecture of complex 3D structures is possible by modifying the staple strands. Using DNA origami, nanopores can be created. Early designs[134] were inspired by □-hemolysin,[102] with a transmembrane stem and a barrel-shaped cap that interacts with the lipid membrane. To overcome the unfavourable interactions between the negatively-charged DNA and the hydrophobic bilayer, 26 cholesterol moieties were attached to the cap. More recent designs have created nanopores with larger diameters, which are suited for functionalising the pore interior or transferring larger biomolecules (Fig. 4D left).[135,136]

Pores consisting of DNA can also be formed without relying on the relatively large size of DNA origami. For example Burns et al. reported the insertion of DNA nanopores based on 6 DNA double helices surrounding a 2 nm pore covalently modified with a hydrophobic belt.[137,138] Conductances of 395 and 250 pS were reported. Göpfrich et al. also reported membrane spanning DNA nanopores, where the pore was lined with 4 duplexes instead of 6, reducing the pore diameter to 0.8 nm.[139] These pores showed multiple conductance states and gating behaviour in single-channel electrophysiology experiments, with a maximum conductance of 300 pS. Ligand-gated DNA origami nanopores (Fig. 4D right) have also been reported, exploiting a lock and key mechanism[140], where a blocking duplex across the channel opens upon binding to two docking sites. Subsequent introduction of a complementary "key" strand unzipped the locking strand, opening up the channel pore.

As an extreme example of a minimal DNA pore, a hydrophobically-functionalized single DNA duplex has also been reported to act as a nanopore.[141] Keyser and co-workers decorated a single DNA duplex with 6 porphyrin tags to allow incorporation in a lipid bilayer, stabilizing a toroidal pore in the membrane. The resulting system had a conductance of 80 pS and showed stochastic gating.

Receptors

As opposed to ions and small molecules, larger signalling molecules do not physically pass through the membrane. Instead, their signals are transferred via interaction with membrane-spanning receptors, where structural changes trigger downstream processes. Key characteristics of receptor-mediated signal transduction include the receptor's affinity to bind ligands, and the amplification of the signal, either directly or via a mediating secondary messenger. Two mechanisms used in nature have inspired researchers to produce artificial transmembrane receptors: (1) ligand-induced oligomerisation of receptor monomers or stabilization of preformed dimers to activate a catalytic site across the membrane, (2) conformational change, where the receptor alters its conformation upon ligand binding.

Receptor oligomerisation

The archetypal example of receptors that function via oligomerisation are receptor tyrosine kinases, which play a crucial role in signal transduction: binding of a ligand to an

extracellular domain either triggers receptor dimerization, or stabilizes a pre-formed dimer, leading to activation of their intracellular kinase domains. The subsequent autophosphorylation of tyrosine residues creates binding sites for specific proteins that then transmit the information further downstream.[142–147]

In the early 90s, Kikuchi and Murakami designed cationic steroid cyclophanes which could recognise anionic guest molecules when placed in an artificial bilayer membrane, with a binding affinity in the high micromolar (μM) range.[148–151] Subsequently in 1998 they demonstrated the activation of copper(II)-inactivated lactate dehydrogenase (LDH) by coordination of copper(II) to steroid cyclophane receptors that had recognised 1-hydroxy-2-naphtaldehyde as a signalling molecule.[152] Although occurring completely on one side of the membrane, this can be considered as a first artificial bilayer signalling system. This approach was later expanded to include receptor dimerization upon ligand binding (Fig. 5A)[153–155] and photo-switchable receptors.[156]

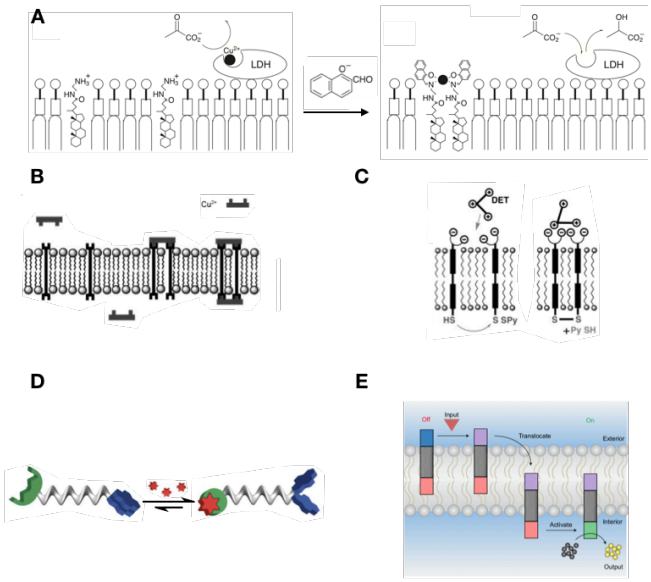

Figure 5. Artificial receptors. (A) First artificial bilayer signalling system. Recognition of 1-hydroxy-naphtaldehyde by the steroid cyclophane receptors results in coordination with copper(II), which activates the copper(II)-inactivated lactate dehydrogenase (LDH).[153] (B) Receptor dimerization due to ligand binding occurs via copper(II) coordination to dansyl ethylenediamine receptor moieties.[159] (C) The release of thiopyridine as a secondary messenger after diethylenetriamine (DET) induced receptor oligomerisation completes the receptor cycle of primary messenger-dimerisation-secondary messenger.[160] (D) G-protein-coupled receptor-like signal transmission is mimicked via transmembrane □-aminoisobutyric acid helices. Binding of chiral carboxylate ligands triggers a change in the helicity, as reported by pyrene fluorescence.[171-173] (E) Transmembrane signal transduction via translocation is achieved via receptor that is too short to span the bilayer. An input signal allows the receptor to migrate to the other side of the bilayer, where secondary reactions can take place.[176] Images reproduced with permission.

A second notable design used membrane-spanning compounds with protected thiol moieties protruding from the bilayer.[157] Deprotection and oxidation of the external thiols

resulted in dimerisation of the receptors via the formation of a disulfide bridge, which in turn lead to the release of pyridine-2-thiol on the other side of the membrane. Although not triggered by a specific primary messenger, this system was the first demonstration of artificial transmembrane signal transduction, where an event on one side of the membrane triggers a reaction on the other side. Subsequent efforts produced systems responsive to a specific primary messenger (Fig. 5B).[158,159] Although the reported system was responsive to a primary messenger, with an affinity in the high μM range, the signalling was not unidirectional across the membrane.

Unidirectional artificial transmembrane signalling was reported by Bernitzki and Schrader in 2009, with an asymmetric transmembrane receptor presenting bisphosphonate dianion headgroups on one side of the bilayer, and a fluorescent moiety on the other. Addition of diethylenetriamine (DET) induced dimerization of two receptors, which in turn changed the fluorescence on the other side of the membrane (Fig. 5C).[160] Secondary messenger release following activation is also possible.[161]

Conformational change

Another mechanism to transduce signals across a membrane is based on biological transmembrane receptors, such as the G-protein-coupled receptor (GPCR) superfamily. Binding of a ligand induces a conformational change in the receptor, propagated across the membrane, resulting in the activation of a receptor-associated proteins, which transmits the signal further downstream.[162–170]

In a bid to achieve this type of signalling using an artificial receptor, Webb and Clayden initially demonstrated that a chiral ligand interacting with an aminoisobutyric acid (Aib) helical foldamer, could induce overall changes in the handedness of the helix, thereby communicating a signal over several nanometers (Fig. 5D).[171–173] Following up on this work, in 2016 this team modified one terminus of the helical foldamer with a photo-responsive azobenzene motif. Upon insertion into a membrane, they illustrated that the light-induced switching of the helical handedness could be propagated across a bilayer.[174] A year later, in 2017, the same group modified the artificial receptor with a binding pocket to interact with primary messengers.[175] The handedness of the helical receptor depended on the chirality of the carboxylic-ligand, which bound tightly to the receptor (K_d < 1 μM), as demonstrated by fluorescence of a pair of pyrenes placed on the other terminus, protruding from the other side of the membrane. This last system illustrates that de novo designed synthetic foldamers can transduce signals by conformational changes, mimicking the working principle of GPCRs, although no secondary messengers were included, nor was signal amplification established.

Membrane translocation

As an alternative to the mechanisms observed in nature, membrane translocation of a synthetic transducer has also been reported to achieve transmembrane signalling (Fig. 5E).[176] Reported by Hunter and Williams, this receptor consists of a membrane-soluble steroid core which does not entirely span the bilayer, and two headgroups that can switch between a polar and a non-polar state. A protonated morpholine headgroup, which is

locked in the aqueous phase at low pH, acts as the external sensor. Raising the pH deprotonates the morpholine moiety, allowing it to enter the membrane, thereby translocating the receptor across the membrane. This movement allows the other headgroup, a neutral pyridine-oxime, to protrude into the aqueous phase on the other side of the membrane. In the aqueous phase, the pyridine-oxime coordinates with zinc(II), which lowers the pKa of the oxime. Subsequent deprotonation activates it as a catalyst for a hydrolysis reaction, resulting in the formation of fluorescent product. The translocation mechanism was shown to be reversible and a modest five-fold signal amplification was reported in a timespan of several hours. Subsequent modifications have incorporated response to a primary messenger[177], and the catalytic production of surfactant as a secondary messenger, which in turn released calcein from the vesicles, resulting in a fifteen-fold downstream signal increase.[178]

Carriers and pumps

Whereas ion channels make use of a selectivity filter to distinguish cargo, carriers bind their solutes and undergo conformational changes when transporting them across the membrane. Additionally, the binding sites for the transported molecules are only accessible on one side of the membrane. Due to the large diversity in carriers and their substrates, transport rates differ substantially. However, as a rule, their transport rates are several orders of magnitude lower than for ion channels. Another key parameter of carriers is their substrate-specificity, which translates into (generally nanomolar) dissociation constants, resembling those of receptors.

Two main families of transporters can be found in nature: the ATP-binding cassette (ABC) superfamily and the solute carrier (SLC) superfamily.[179] ABC transporters are primary active transporters, which overturn ATP using highly conserved ABC domains in transporting substrates against their concentration gradient into or out of the cell.[180,181] They are important players in e.g. antigen processing and exogenous substrate export.[182,183] Prokaryotes employ ABC transporters in both the export of noxious substances, and nutrient uptake.[184] ABC transporters also play a key role in multidrug resistance in bacteria, and in human cancer.[185,186] Most ABC transporter rates are determined via ATP hydrolysis assays, making it difficult to assess the number of individual molecules being transported over time. However, to illustrate the much lower rate of transport, Zollmann et al. have analyzed peptide transport through the peptide ABC transporter TAPL into single liposomes and reported a rate of only eight molecules per minute.[187]

SLCs are primarily used in the selective uptake of small molecules, ranging from (in)organic ions and urea, to amino acids.[188–191] Transport can occur either passively, down a concentration gradient, or actively if the SLC uses electrochemical gradients to transport its substrates against their concentration gradient.[192]

Pumps transport their substrates against a concentration gradient by harnessing ATP hydrolysis or the co-transport of other ions along their electrochemical gradient.[193–195] The cardinal example P-type ATPase[194,195] is the Na+/K+-pump, which maintains the cell membrane potential by transporting Na+ out of and K+ into the cell. Alternatively, the

rotary molecular motor F0F1 ATP (synth)ase is the primary source of ATP production in the cell, coupling a proton gradient to ATP synthesis, or establishing a proton gradient upon ATP consumption.[196,197]

The systems mentioned in this section were classified as carriers due to an explicit conformational change, or movement through the membrane to pick up cargo, thereby demonstrating a different mode of action than the artificial ion channels. Like artificial ion channels, the field of artificial transporters has long been dominated by organic and inorganic supramolecular chemistry. Many excellent reviews are present in the literature discussing these extensively[27,32,198–201], so we will only provide a few illustrative examples: in 2002, Smith and colleagues rationally designed crown-based macrobicycle carriers capable of co-transporting NaCl or KCl across lipid membranes via diffusion across the bilayer.[202] Transport was proposed to occur via an uncomplexed carrier diffusing into the vesicle, where it coordinated with the salt, and subsequent diffusing of the carrier-salt complex out of the vesicle. Molecular 'umbrellas', which attach a central substrate-binding scaffold to two amphiphilic components that shield the substrate from the environment, have been shown to selectively transport ATP over glutathione across membranes (Fig. 6A).[203,204] More recently, inspired by biomolecular machinery, a rotaxane molecular shuttle was developed that could transport K+ across lipid membranes (Fig. 6B).[205] The shuttle is composed of an amphiphilic molecular thread with three binding stations, via which a macrocycle tethered to a K+ carrier could translocate from one side of the bilayer to the other. This last example illustrates the interest in developing bio-inspired molecular machines.[206,207]

True artificial transmembrane machines should be capable of actively transporting substrates against their electrochemical gradient by consuming another type of energy. To this extent, two notable examples should be mentioned that realized the build-up of a transmembrane proton gradient by using light as an energy source. The first mimicked the photoinduced electron transfer that occurs during photosynthesis[208] using a molecular 'triad' consisting of an electron donor and acceptor linked to a photosensitive porphyrin group in a membrane to generate a proton gradient. The authors successfully coupled this gradient to F0F1 ATP (synth)ase to drive ATP production.[209] A similar result was obtained in the second example, when the Matile group demonstrated that p-octiphenyl rods bearing fluorescent naphthalene-diimides self-assembled in lipid bilayers to form a π-stacked helical structure.[210] In the presence of an electron donor and acceptor system, irradiation of the transmembrane structure resulted in long-lived charge separation across the membrane, which could be neutralized by quinone-coupled proton transport, resulting in a proton gradient. Finally, they showed that they could open up the helix by adding an intercalator, resulting in the formation of an ion channel, which quickly dissipated the proton gradient.

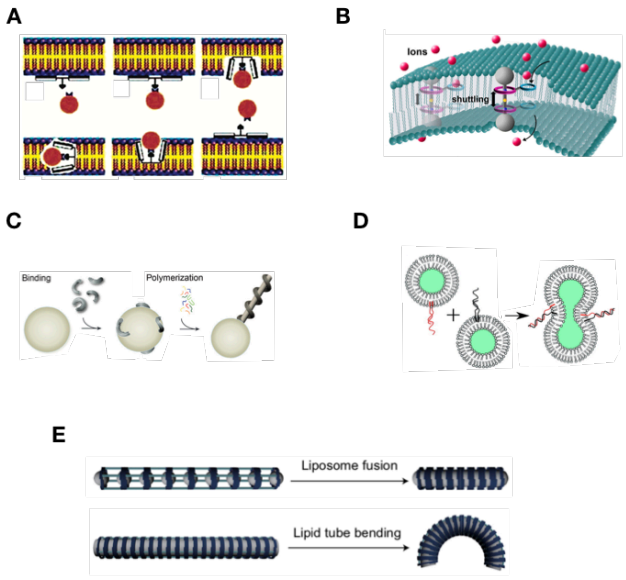

Figure 6. Transporters & physical properties of the membrane. (A) Proposed mechanism of a molecular umbrella design that selectively transports ATP over glutathione across a lipid bilayer. Top left to bottom right: binding of hydrophilic cargo to the umbrella is followed by a conformational change in the scaffold to shield the cargo upon bilayer insertion, and finally re-opening of the umbrella to release the cargo on the other side of the membrane.[203] (B) A rotaxane consisting of an amphiphilic thread and a macrocycle wheel, which is tethered to a crown ether, shuttles back and forth through the membrane to transport K+ ions.[205] (C) Polymerization of DNA origami curls into long helical structures induces and stabilizes lipid tubules in GUVs. The DNA “springs” simultaneously pull at the lipid membrane, evoking tubule formation, and hold the latter in place when formed.[225] (D) Liposome fusion can be achieved using DNA hybridization, effectively mimicking the operation of SNARE proteins.[231] (E) DNA origami nanocages can also trigger vesicle fusion. Vesicle-bearing nanocages are brought together by removing the connecting pillars via toehold-mediated strand displacement (top). Upon the creation of a lipid tubule, complex structures can be enforced by manipulating the geometry of the DNA nanocages (bottom).[234] Images reprinted with permission.

Physical properties of the membrane

Cells are also capable of the collective reorganization of the membrane itself, both at the nano- or micro-scale. Changes in the shape, composition, and phase of lipid membranes is mediated by a wide range of important proteins. In turn, changes in these lipid properties are sensed by a second cohort of transmembrane and membrane associated proteins. Mimicking these processes might be an alternative mechanism for signal transduction.

Curvature

Protein association with the membrane can be sufficient to cause significant shape changes[211–213], either for example through templating in proteins (such as BAR domains[214] or in clathrin-coated pits[215]) or through molecular crowding.[216] Such shape changes are sufficient to cause membrane tubulation[217], fission[218], and endo- or

exocytosis.[219–222] Whereas it is straightforward to understand how endocytosis permits information transfer across the membrane, shape changes without cargo transfer are harder to envisage as a mechanism of signal transduction. However, biology does exploit such mechanisms, as proteins that sculpt membrane curvature can also act as curvature sensors.[223]

Very recently, Schwille and co-workers designed three DNA origami scaffolds with varying degrees of curvature to mimic various BAR dimers.[224] They reported binding of the scaffolds to model lipid membranes via linkage to a cholesterol moiety, which in turn induced membrane deformation and tubulation in giant unilamellar vesicles (GUVs). Alternatively, Grome et al. demonstrated that DNA nanosprings – obtained by polymerizing curled DNA origami – could also induce membrane tubulation (Fig. 6C).[225] The DNA nanosprings are proposed to pull on the lipid as they elongate, and subsequently hold the tubule in place. Like the BAR-mimicking DNA scaffolds, it was shown that a higher surface coverage and lower membrane tension enhanced tubulation. As opposed to mimicking BAR domain proteins, this last design was inspired by dynamins and ESCRT machineries, whose helical structure upon polymerization coats lipid tubules.[226,227] Combined, these two works illustrate that the field of DNA origami has matured into being able to make sophisticated structures that can mimic natural protein assemblies used in membrane deformation.

Vesicle fusion

Synaptic vesicle fusion[228–230], which employs highly specialized SNARE proteins, has been mimicked by using DNA hybridization as the driving force. Anchored complementary DNA strands in bilayers of different vesicles have been shown to induced lipid mixing and vesicle fusion (Fig. 6D).[231] Fusion efficiency is dependent on linker sequence and length.[232] Alternatively, DNA origami rings[233] and nanocages (Fig. 6E)[234] have also been used to facilitate fusion of liposomes.

Demixing

Taking inspiration from the lipid raft hypothesis, [235] phase changes within the membrane can also alter the spatial organisation of membrane components, and hence communicate a signal across the membrane. Although not yet realized as components of an artificial cell, such processes are well developed; for example, crosslinking of the ganglioside GM1 causes lipid demixing in GUVs.[236] DNA origami is one obvious tool with which to alter membrane interactions, and have been reviewed recently.[237] Clearly there is the potential for strong coupling between factors affecting the physical properties of the membrane, for example between lipid demixing and curvature.[238]

Conclusions

Drawing inspiration from nature, there is a veritable cornucopia of artificial systems capable of transmitting signals across a membrane. Functioning artificial systems have been demonstrated that duplicate the function of almost all classes of biological signal transduction across the membrane. However, despite these longstanding efforts we

admittedly still fall far short of the capabilities of the biological systems we seek to mimic. Notably, in comparison with biology, the timescales for most of these artificial systems are slow, their selectivity is sub-optimal, and they lack the ability to respond to more than one type of stimulus, or to amplify a molecular signal. It is unlikely we can outcompete nature in terms of catalytic turnover frequency, but we can strive to construct mimics that have a comparable degree of selectivity and complexity.

There is also much to learn from other aspects of synthetic biology, where standardization of inputs and outputs from these processes would permit coupling into more complex and ambitious artificial function.

Finally, it is worth noting that here we focus only on one aspect of Gánti's checklist for artificial life; the processes reviewed here must be combined with other key features. Especially the coupling to metabolic and/or reproductive systems should be a prime objective to enable the construction of more advanced systems.

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