COMMENTARY

Design of energy-transducing artificial cells

James P. Allen[a,1]

A sustainable society requires sources of renewable energy that are efficient, cost-effective, and robust. Achieving such energy sources represents a significant challenge that requires the development of novel technologies, including the creation of materials that control physical and chemical transformations at a molecular level. Photosynthesis performs solar energy conversion using Earth-abundant metals, a broad spectral range, and materials that operate under ambient conditions. The components of photosynthetic systems work cooperatively and efficiently with enviable rates, providing the motivation for using these components in energy transduction strategies. In PNAS, Altamura et al. (1) move us closer toward this goal by demonstrating how protein complexes from photosynthetic bacteria can be incorporated into artificial cells to convert light into chemical energy, in the form of a proton gradient, using new technical approaches that should be of general applicability.

Since the pioneering work of Mitchell (2), it has been recognized that organisms use differences in proton concentrations across the cell membrane, termed proton gradients, to perform energy transduction. Biochemical reactions are coupled with the transfer of protons across the membrane through the use of proteins present in the membrane. For example, the membrane protein ATP synthase uses proton transfer to form the energy-rich compound ATP from ADP. In bacterial photosynthesis, the formation of the proton gradient is driven by the absorption of light by a pigment–protein complex called the reaction center (3). The reaction center is a membrane protein containing a number of cofactors arranged in two branches that span the membrane (Fig. 1). Light energy is absorbed by the reaction center followed by the transfer of an electron across the membrane from the electron donor through one of the branches to a quinone acceptor. After the absorption of a second photon, two protons are transferred from the cytoplasmic side to the doubly reduced quinone, forming a quinol. The electron and proton transfer reactions are connected with another membrane protein, the cytochrome bc_1 complex. In this complex, electron and proton transfer converts the quinol back into a quinone in a process that is coupled with the release of protons to the periplasmic side of the membrane. Thus, protons are transferred across the membrane, resulting in light energy being effectively transformed into stored energy in the form of a proton gradient.

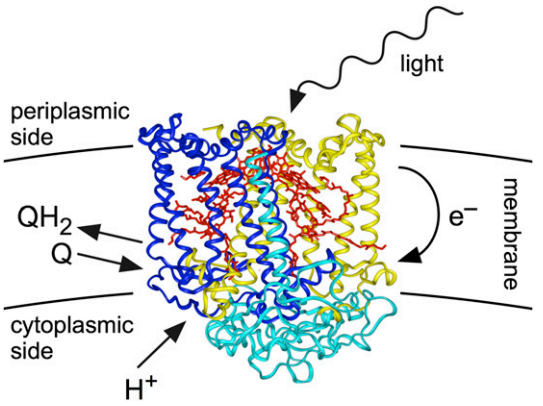

Fig. 1. Three-dimensional structure of the reaction center from *Rhodobacter sphaeroides*. The highly hydrophobic L (yellow) and M (blue) subunits, each of which contains five transmembrane helices, are largely buried within the cell membrane, whereas the hydrophilic H subunit (cyan) largely resides in the cytoplasm, except for the single transmembrane helix. Light absorption by the reaction center results in the vectorial transfer of an electron among the cofactors (red) from the periplasmic to the cytoplasmic sides of the reaction center. After absorption of a second photon, the doubly reduced secondary quinone picks up two protons from the cytoplasmic space, forming the quinol.

For many years, scientists have worked to recreate the energy transduction abilities of photosynthetic organisms by embedding reaction centers within artificial membranes. Early experiments had shown that the bacterial reaction center in artificial planar lipid bilayers and liposomes could absorb light and transfer electrons (4–6). Reaction centers in lipid bilayers supported on glass surfaces are stable with the advantage of the bilayer being fluid over a large area (7). The spherical structure of liposomes provides an inner solvent region that can have a different proton concentration than the outside solvent, thus allowing the opportunity to

[a]School of Molecular Sciences, Arizona State University, Tempe, AZ 85287
Author contributions: J.P.A. wrote the paper.
The author declares no conflict of interest.
See companion article on page 3837.
[1]Email: jallen@asu.edu.

www.pnas.org/cgi/doi/10.1073/pnas.1703163114

produce a proton gradient. Although the reaction centers could be functionally added into the membranes, a noticeable disadvantage of many of the liposome studies was the incorporation of the reaction centers in both orientations, in which case light absorption results in electron and proton transfer in both directions and no net transfer. A preferential orientation was achievable with the use of a narrow range of conditions, such as using charged lipids at specific ionic strengths (8).

In PNAS, Altamura et al. (1) describe a novel system that combines the advantages of reaction centers preferentially oriented in one direction with the ability to produce light-generated proton gradients. An innovation compared with the previous studies is their use of giant unilamellar vesicles that contain the reaction centers within the membrane (9, 10). Giant unilamellar vesicles possess bilayer membranes formed from a variety of natural lipids, but their size is significantly larger than that of liposomes. In this report, the giant unilamellar vesicles had an average diameter of 20 μm compared with typical diameters of less than 0.1 μm for liposomes. With the giant unilamellar vesicles, the insertion of the reaction centers was found to be technically straightforward and resulted in the proteins being functional, with nearly all (90%) being oriented in one direction. The relatively large size of the vesicles provided direct opportunities to investigate their properties using confocal microscopy at room temperature, which is not feasible for the smaller liposomes. Fluorescence measurements determined the localization of the reaction centers within the vesicle membranes and quantified the light-induced increase of the pH of the interior space, corresponding to formation of a proton gradient.

The work of Altamura et al. (1) provides a demonstration of the feasibility of creating artificial cells with novel functions driven by light and proton gradients. The inclusion of the F_0F_1-ATP synthase and cytochrome bc_1 complex with the reaction center should result in an artificial cell that could convert light energy into proton gradients that then would use the gradient to drive the conversion of ADP into ATP. The ability of the system to absorb light would be limited by the spectral response of the reaction center. Enhanced solar energy conversion efficiencies could be achieved by reengineering the properties of the reaction center to better match the overall energetics of the solar spectrum (11). The reaction center has proven to be robust to manipulations that alter the electronic structures of the bacteriochlorophyll cofactors and hence the light region that they can absorb (12). In addition, the use of bacterial reaction centers capable of binding Mn clusters could potentially allow water to be used as the electron donor as occurs in photosystem II in plants. Alternatively, the functionality of such cells could be expanded with the use of molecular complexes capable of electron and proton transfer in place of the reaction center, as has been demonstrated in liposomes (13). The creation of bioinspired artificial cells that mimic photosynthetic cells by using light and protein gradients to drive chemical reactions would represent a self-sustaining system. These systems could potentially be incorporated with photovoltaics to develop novel solar energy conversion devices.

More long term, artificial cells capable of energy transduction could be used as power sources to drive nanoscaled artificial molecular machines. The design of artificial cells with proteins embedded in the membrane as well as confined in the interior of the vesicles opens the door to creating systems capable of performing multistep reactions involving multiple proteins. The reactions could be enzymatic and result in chemical changes but also mechanical motion (14). In addition to the use of natural proteins, the field of de novo protein design is moving from building proteins similar to those existing in nature to crafting ones with novel functions that are tailored to address specific functions (15). Coupling novel protein designs into vesicles with multiple components provides the opportunity to develop new chemical platforms capable of controlling complex chemical reactions.

Acknowledgments

J.P.A.'s research is supported by National Science Foundation Grant CHE 1505874.

1 Altamura E, et al. (2017) Highly oriented photosynthetic reaction centers generate a proton gradient in synthetic protocells. *Proc Natl Acad Sci USA* 114:3837–3842.
2 Mitchell P (1979) Keilin's respiratory chain concept and its chemiosmotic consequences. *Science* 206:1148–1159.
3 Allen JP, Williams JC (1998) Photosynthetic reaction centers. *FEBS Lett* 438:5–9.
4 Pachence JM, Dutton PL, Blasie JK (1979) Structural studies on reconstituted reaction center-phosphatidylcholine membranes. *Biochim Biophys Acta* 548:348–373.
5 Schönfeld M, Montal M, Feher G (1979) Functional reconstitution of photosynthetic reaction centers in planar lipid bilayers. *Proc Natl Acad Sci USA* 76:6351–6355.
6 Overfield RE, Wraight CA (1980) Oxidation of cytochromes *c* and c_2 by bacterial photosynthetic reaction centers in phospholipid vesicles. 1. Studies with neutral membranes. *Biochemistry* 19:3322–3327.
7 Salafsky J, Groves JT, Boxer SG (1996) Architecture and function of membrane proteins in planar supported bilayers: A study with photosynthetic reaction centers. *Biochemistry* 35:14773–14781.
8 Venturoli G, Melandri BA, Gabellini N, Oesterhelt D (1990) Kinetics of photosynthetic electron transfer in artificial vesicles reconstituted with purified complexes from *Rhodobacter capsulatus*. I. The interaction of cytochrome c_2 with the reaction center. *Eur J Biochem* 189:105–112.
9 Chan YHM, Boxer SG (2007) Model membrane systems and their applications. *Curr Opin Chem Biol* 11:581–587.
10 Walde P, Cosentino K, Engel H, Stano P (2010) Giant vesicles: Preparations and applications. *ChemBioChem* 11:848–865.
11 Blankenship RE, et al. (2011) Comparing photosynthetic and photovoltaic efficiencies and recognizing the potential for improvement. *Science* 332:805–809.
12 Olson TL, Williams JC, Allen JP (2013) Influence of protein interactions on oxidation/reduction midpoint potentials of cofactors in natural and de novo metalloproteins. *Biochim Biophys Acta* 1827:914–922.
13 Steinberg-Yfrach G, et al. (1998) Light-driven production of ATP catalysed by F_0F_1-ATP synthase in an artificial photosynthetic membrane. *Nature* 392:479–482.
14 Erbas-Cakmak S, Leigh DA, McTernan CT, Nussbaumer AL (2015) Artificial molecular machines. *Chem Rev* 115:10081–10206.
15 Huang PS, Boyken SE, Baker D (2016) The coming of age of de novo protein design. *Nature* 537:320–327.