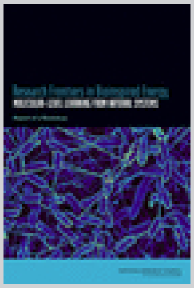

Research Frontiers in Bioinspired Energy: Molecular-Level Learning from Natural Systems: A Workshop

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Research Frontiers in Bioinspired Energy
MOLECULAR-LEVEL LEARNING FROM NATURAL SYSTEMS

Report of a Workshop

Committee on Research Frontiers in Bioinspired Energy

Board on Chemical Sciences and Technology

Division on Earth and Life Studies

NATIONAL RESEARCH COUNCIL
OF THE NATIONAL ACADEMIES

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Preface

In May 2007, the National Academies Chemical Sciences Roundtable held a public workshop on the topic of *Bioinspired Chemistry for Energy*,[1] where government, academic, and industry representatives discussed promising research developments in solar-generated fuels, hydrogen-processing enzymes, artificial photosynthetic systems, and biological-based fuel cells. Workshop participants identified the need for a follow-up activity that would explore bioinspired energy processes in more depth and involve a wider array of disciplines as speakers and participants. Particularly, workshop participants stressed the importance of holding a workshop that would include more researchers from the biological sciences and engineering, as well as those involved in technological advances that enable progress in understanding these systems.

Building upon the 2007 workshop, the National Academies Board on Chemical Sciences and Technology convened the workshop described in this report, titled *Research Frontiers in Bioinspired Energy: Molecular-Level Learning from Natural Systems.* The workshop featured invited presentations and included discussion of key biological energy capture, storage, and transformation processes, gaps in knowledge and barriers to transitioning the current state of knowledge into applications, and underdeveloped research opportunities that might exist beyond disciplinary

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[1] National Research Council. 2008. *Bioinspired Chemistry for Energy: A Workshop Summary.* Washington, DC: National Academies Press.

boundaries. Presentations and discussions focused on molecular-level understanding rather than development of large-scale applications.

While reading this document, we sincerely hope you will come across a statement, figure, or discussion topic that entices you to collaborate or to interact with researchers in other disciplines or sectors in the area of bioinspired energy. Although not comprehensive, this report should provide a good overview of some of the exciting and broad ranges of approaches scientists and engineers are exploring at the interfaces of chemistry, biology, geology, engineering, and energy applications.

The Committee on Research Frontiers in Bioinspired Energy:
Molecular-Level Learning from Natural Systems

Acknowledgment of Reviewers

This workshop summary has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published summary as sound as possible and to ensure that the summary meets institutional standards for clarity, objectivity, and responsiveness to the charge. The review comments and draft manuscript remain confidential to protect the integrity of the process. We wish to thank the following individuals for their review of this workshop summary:

Steven Chuang, University of Akron
Sharon Haynie, E. I. du Pont de Nemours & Company
Charles E. Kolb, Aerodyne Research, Inc.
Michael Ladisch, Purdue University
Nikolai Lebedev, Naval Research Laboratory
Frances S. Ligler (National Academy of Engineering), Naval Research Laboratory

Although the reviewers listed above have provided many constructive comments and suggestions, they did not see the final draft of the workshop summary before its release. The review of this summary was overseen by **Marye Anne Fox** (National Academy of Sciences), University of California, San Diego. Appointed by the National Research Council, she was responsible for making certain that an independent examination of this summary was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this summary rests entirely with the authors and the institution.

Contents

Appendixes

1

Introduction

> *Energy is something that biology has been doing very successfully for many billions of years. In fact, it underpins the whole notion of an effective biological system on the planet.*
>
> –Penelope Boston

> *It is difficult to define the destination before the science and technology can be developed.*
>
> –Janet Westpheling

> *What I cannot create, I do not understand*
>
> –Richard Feynman

Is it possible that an ancient microbe, in existence for millions of years, could hold the key to making low-cost and efficient devices for future supplies of clean water? Are there patterns in protein structure that provide clues for creating artificial photosynthetic systems that are more robust and efficient than those found in nature? Can sulfur-loving bacteria living in hydrothermal vents at the bottom of the ocean provide new ways to capture and utilize methane (i.e., low-energy oxidation of methane to methanol)? As current energy sources are dwindling and the demand for energy is expected to more than double by 2050 (U.S. Department of Energy, 2005), the development of alternative sources and approaches to energy is needed. Over billions of years, biological organisms have evolved and optimized methods to create and harness energy through photosynthesis, chemosynthesis, and basic cellular processes. The underlying mechanisms by which organisms produce energy have provided researchers with a template from which they try to mimic the processes, or to inspire new techniques for producing alternative energy technologies to address society's long-term energy needs.

Building upon a 2007 workshop (National Research Council, 2007), the National Academies Board on Chemical Sciences and Technology convened the Committee on Research Frontiers in Bioinspired Energy to organize a second workshop in 2011 which, according to the statement of task, would explore the molecular-level frontiers of energy processes in nature through an interactive, multidisciplinary, and public format.[1] Specifically, the committee was charged to feature invited presentations and include discussion of key biological energy capture, storage, and transformation processes; gaps in knowledge and barriers to transitioning the current state of knowledge into applications; and underdeveloped research opportunities that might exist beyond disciplinary boundaries. This report is an account of what occurred at the workshop, and does not attempt to present any consensus findings or recommendations of the workshop participants. It summarizes the views expressed by workshop participants, and while the committee is responsible for the overall quality and accuracy of the report as a record of what transpired at the workshop, the views contained in the report are not necessarily those of the committee.

WORKSHOP ORGANIZATION

Opening remarks were made by workshop organizing committee member Douglas Ray, from Pacific Northwest National Laboratory, followed by an opening plenary talk presented by Leslie Dutton of the University of Pennsylvania. Dutton's work has revealed common machinery in enzymes driven by electron transfer, which he has used to construct synthetic enzymes and hopes can be applied to helping to meet needs in energy and medicine. The subsequent technical sessions of the workshop focused on energy transformations, energy capture, and bioinspired energy systems, and are briefly described below. Greater details about each speaker's presentation can be found in Chapter 2. A summary of breakout session discussions is given in Chapter 3.

Energy Transformations

This session began with **Penelope Boston**, from the New Mexico Institute of Mining and Technology, speaking about her work in extreme environments of the subsurface of Earth. Boston described microbes from these environments that carry out chemical processes in unique and novel ways with relevance to energy applications. Next, **Steven Benner**, from the Foundation for Applied Molecular Evolution, spoke about his research in the synthetic biology field. One of the goals of his work is to develop a

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[1] See Appendixes A–D for the committee's statement of task, the workshop agenda, organizer and speaker biographies, and participant list, respectively.

system that is self-sustaining and capable of evolving. Following the first session, workshop attendees participated in the first breakout session.

Energy Capture

In this session, **Janos Lanyi,** of the University of California, Irvine, presented his research on bacteriorhodopsin (BR)—the light-driven electrogenic ion pump in the cytoplasmic membrane of *Halobacterium salinarum.* He highlighted the key principles he has learned from BR that are needed to construct useful energy systems, especially those involving hydrogen transport. **Rudolf Thauer,** from Max Planck Institute, then presented data on anaerobic oxidation of methane with sulfate as an electron acceptor in microorganisms. Thauer discussed how the mechanism used by those microorganisms could inspire chemists to build catalysts that might be used to oxidize methane to methanol. Following the energy capture session, participants were asked to attend the second breakout session.

Evening Plenary Session

The evening plenary talk was given by **Marian Plotkin** from the University of Singapore. Plotkin described his research on Oriental hornets and solar energy–correlated digging activity. He discussed in detail how the pigments and molecular structure of the hornet cuticle exhibit useful antireflection and light-trapping properties.

Bioinspired Energy Systems

On the second day of the workshop, **Kenneth Nealson**, from the University of California and the J. Craig Venter Institute, opened the session on bioinspired energy systems. Nealson discussed his research on the microorganism *Shewanella,* which has unique redox properties. He highlighted some promising results of using the microbe for fuel cells and water purification. **Felisa Wolfe-Simon**, National Aeronautics and Space Administration astrobiology fellow at the U.S. Geological Survey, spoke next. She highlighted her research on exploring photosynthesis in cyanobacteria that do not make oxygen and about research she published on a microbe that can sustain its growth in normally biologically toxic levels of arsenic. Both Nealson's and Wolfe-Simon's projects highlighted the inherent flexibility of biology. After the session, participants were asked to join the third, and final, breakout session for discussion.

Plenary Session

The final speaker at the workshop was **Nadrian Seeman** from New York University. Seeman described how DNA's chemical information can be used for bottom-up nanoscale control and to create nanoscale mechanical devices. The workshop concluded with remarks by organizing committee member **James C. Liao** from the University of California, Los Angeles.

Breakout Sessions

Workshop participants met in three different breakout discussion groups during the course of the workshop. Each topical session included a breakout discussion and report-back time. The breakout sessions allowed for more in-depth and interactive discussion between participants during the workshop. The composition of the discussion groups was multidisciplinary and provided feedback to the larger group on

- Key issues raised or important information provided by the guest speakers,
- Research opportunities, especially for interdisciplinary collaborations, and
- Resource and educational needs to support long-term advances.

There were five groups for each breakout session (14–16 participants per group). The composition of each group changed each session to ensure maximum mixing of workshop participants. Each discussion group was run by an assigned discussion leader and a committee member. The discussion leaders were as follows (biographies in Appendix C):

- **Judy Wall**, University of Missouri, Columbia
- **Tom Moore**, Arizona State University
- **Janet Westpheling**, University of Georgia
- **R. David Britt**, University of California, Davis
- **Robert Kelly**, North Carolina State University

Discussion leaders were responsible for posing questions and facilitating discussion. Committee members were responsible for ensuring that the discussion stayed on task and within scope of the workshop objectives, and for capturing key ideas from the discussion (summarized in Chapter 3). Each breakout session began by considering the following set of questions:

- What were the key issues raised or important information provided by the guest speakers in the plenary session?
- Were there any new insights about the ways life forms capture, transform, and store energy at the molecular level?
- How might new and existing information be applied in new ways—to create new energy systems?
- What are the unique perspectives that each discipline brings to studying these processes and systems?
- What are the research opportunities to advance the science, especially for interdisciplinary collaborations?
- What are resource and educational needs for supporting long-term advances in bioinspired energy?

Some of the key issues discussed by breakout group participants during the workshop included defining the term "bioinspired"; exploring microbial diversity and setting priorities; developing and supporting research and collaborative models; supporting current and creating new opportunities for interdisciplinary education, training, and outreach; understanding microbial nanowires and fuel cells; understanding and applying synthetic biology to energy applications; and keeping the big picture in mind.

ONLINE COMPONENT

In trying to make the workshop material readily available to the public, the Board on Chemical Sciences and Technology developed a hub for information relating to the ***Research Frontiers in Bioinspired Energy: Molecular-Level Learning from Natural Systems*** workshop. At the time of this publication, additional material relating to the workshop could be found at *http://dels.nas.edu/global/bcst/bioinspired-energy*. It includes speaker presentations, post-workshop interviews of speakers, and YouTube videos from speakers. Post-workshop interviews asked participants to briefly summarize the research they presented at the workshop and one key issue they believe was raised during the workshop. The YouTube videos showed workshop participants addressing the following questions:

- Why are biological systems useful models for developing new sources of energy?
- What can we learn about energy from biological systems?
- What is one example of an energy device or technology that was inspired by a biological system?

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2

Summary of Speaker Presentations

TIME AND ENERGY SCALES IN BIOLOGY

> *Everything is driven by electron transfer. In biology, in our own systems, every system is rooted, built upon electron transfer.*
>
> – Leslie Dutton

Leslie Dutton, University of Pennsylvania, has spent his research career trying to understand fundamental oxidation-reduction reactions and coupled events in biological systems. He noted that 26 percent of natural enzymes—proteins that catalyze chemical reactions—are engaged in oxidation-reduction reactions. Through the capture of sunlight or chemicals, these reactions are the basis of life on Earth. Enzymes are involved in producing oxygen and food, and are also involved in breaking food down to produce energy and cellular material in humans, animals, and plants. Dutton's work has revealed common machinery in enzymes driven by electron transfer (Moser et al., 1992). He has developed a set of engineering guidelines for how the machinery works and how synthetic proteins that might be able to carry out programmed enzyme functions can be constructed in laboratories (Page et al., 1999).

For example, Dutton has identified common machinery in natural oxidoreductases, which are involved in physiological processes such as photosynthesis. In these enzymes, electrons tunnel one at a time through the insulating protein medium that separates one redox center from another. Dutton has found that almost all (greater than 97 percent) physi-

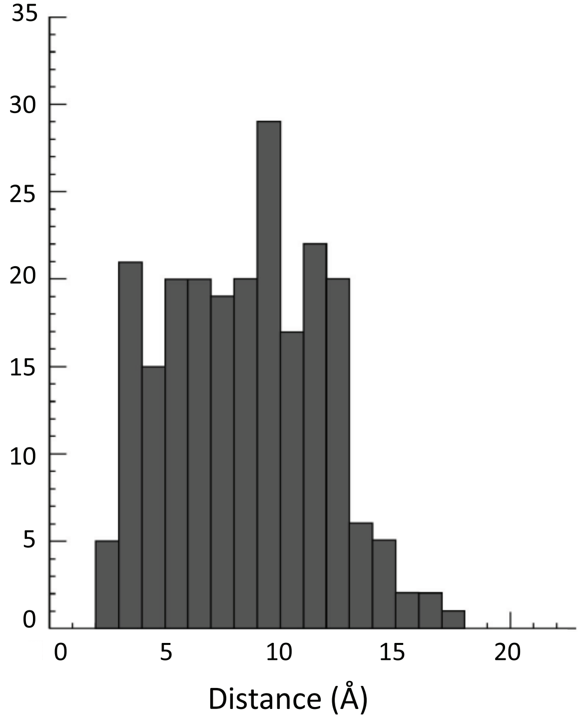

FIGURE 2-1 Distribution of physiologically productive single-electron tunneling distances (in angstroms or Å) in electron transfer and oxidoreductase proteins. More than 97 percent of the proteins have tunneling distances of less than 14 Å.
SOURCE: Adapted from Moser et al. (2010, Fig. 1A).

ologically productive electron reactions in these enzymes have tunneling distances within 4 to 14 Å (Figure 2-1; Moser et al., 2010). These distances appear to be ideal for burying and protecting redox centers so that they can maintain catalytic rates (on the millisecond timescale) found in most physiological enzymatic reactions.

Natural energy systems have evolved a general and simple engineering for electron transfer that can be used for understanding uncharacterized, naturally occurring oxidoreductases, and in constructing synthetic enzymes. Dutton talked about his ultimate goal to design artificial proteins, or what he calls "maquettes," from scratch. He starts with a simple artificial protein scaffold, and then introduces function by progressive, iterative, and entirely reversible design steps. He tries to avoid mimicry of natural enzymes (examples shown in comparison to a maquette in

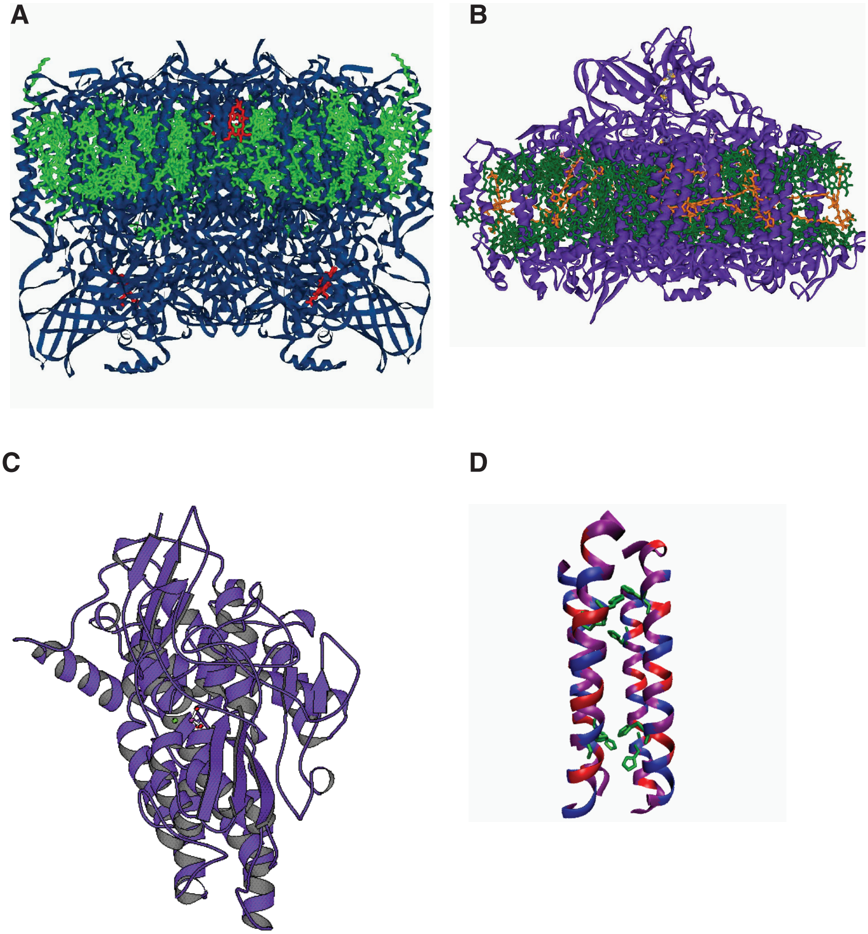

FIGURE 2-2 Comparison of natural enzymatic systems and Dutton's synthetic "maquette": (A) photosystem II, (B) photosystem I, (C) natural hydrogenase, (D) maquette.
SOURCE: Leslie Dutton, presentation slides.

Figure 2-2) and stays independent of natural selection. Dutton hopes to develop general-utility "omnibus" maquettes with closely related structures and diverse functions. He said these synthetic enzymes could be applied to helping meet energy needs, or in medical and clinical settings to replace systems in the human body that are not functioning properly. For example, such a maquette could provide a photocatalytic function for solar energy applications.

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MICROBIAL DISCOVERY

> *Energy is something that biology has been doing very successfully for many billions of years.*
>
> – Penelope Boston

Penelope Boston, New Mexico Institute of Mining and Technology, conducts research on cave geomicrobiology, microbial life in highly mineralized environments, unique or characteristic biominerals, and biosignature detection (Boston et al., 2001). Although her work is not specifically focused on bioinspired energy, Boston's work explores extreme environments, specifically subsurface earth. The biological systems she has encountered serve as inspiration for discovering new approaches to energy tranformations (Figure 2-3). Extreme environments contain biological organisms that are usually novel and unique in many ways. The chemical processes that microbes carry out in subsurface earth is of great interest to researchers seeking to relate biology to usable energy for civilization. Researchers ask questions such as "What are the natural microbial processes that occur in exotic organisms?" and "How can we use what we learn [about the microbes] in industrial applications?"

Boston's work has recently focused on lava tubes, a byproduct of volcanic activity. Lava tubes are found throughout the Earth, and they tend to be heavily colonized by unusual bacteria, fungi, and archaea. Boston noted that the physical conditions to which surface-living organisms are commonly exposed are radically different from those of the interior (i.e., subsurface) conditions of this planet. The biggest difference between the surface level and the interior of the planet is that there is no sunlight in the subsurface, and thus no photosynthesis beyond the entrance zone or twilight zone of the cave. Some of the advantages of studying in these sites are that the temperature is virtually constant throughout the system, the microbes are low-nutrient and mineral-rich, and these sites do not have surface weather, and so they remain isolated and untouched. Part of Boston's work focuses on subsurface biominerals, that is, minerals associated with some biological process, such as sulfur (Boston et al.,

FIGURE 2-3 Slimy strings of bacteria often called “snottites” are found in caves Boston has explored. This cluster was found in Cueva de Villa Luz, Tabasco, Mexico.
SOURCE: Photo copyright Kenneth Ingham. Used by permission.

2006). The minerals found in these environments are clues to what type of metabolism sustains the microbial communities (Melim et al., 2009).

Boston believes that the subsurface rock habitats are the sites for novel metabolism shopping; they are underexploited in terms of interesting characteristics, because very few people have studied these habitats. During the presentation, Boston highlighted her work and that of others, including work in Lechuguilla Cave in New Mexico. The main message Boston made is that if one is in search of novel microbes and novel metabolism, the subsurface is a great place to look.

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SYNTHETIC BIOLOGY

> *If you understand electron transfer, you ought to be able to build your own electron transfer stuff.*
>
> –Steve Benner

Steven Benner, Distinguished Fellow at the Foundation for Applied Molecular Evolution, is interested in chemical genetics, synthetic biology, paleogenetics, planetary biology, systems biology, and the connection of natural history to the physical sciences. Benner introduced four approaches to understanding life: prebiotic chemistry, searching the cosmos, paleogenetics, and synthetic biology. However, the focus of his presentation was his current work on synthetic biology (Benner and Sismour, 2005), which he defined as an “artificial self-sustaining chemical system capable of Darwinian evolution.” As a field, the term synthetic biology was created in 1986 by Waclaw Szybalski (1974) . As discussed by Benner, different risks are involved and need to be considered when studying synthetic biology. The goal of his laboratory is to develop a system that is self-sustaining and capable of evolving, using a six-letter artificial genetic alphabet based on natural nucleic acids, which they refer to as an artificially expanded genetic information system (AEGIS; Yang, et al., 2010).

Benner explained the relationship of the artificial bases to the naturally occurring Watson-Crick nucleic acid base pairs adenine (A) and thymine (T), and guanine (G) and cytosine (C), shown in the top frame of Figure 2-4. He explained that the two principles working in the Watson-Crick base pairing are the size complementary principle and the hydrogen-bonding complementary principle. If the Watson-Crick–based pairing is shifted, to hydrogen-donor-and-acceptor groups within the context of size complementary, eight additional bases can be created, forming four additional base pairs. The artificial base pairs have been named K and X, Z and P, V and J, and iso-C and iso-G.

Although the following results have not been published, according to Benner, he and his collaborators have successfully created synthetic DNA that replicates in artificial cells and is capable of Darwinian evolution. He explained that such synthetic biological systems are ideal and desirable because they do not carry around the billion years of "baggage" like natural systems (i.e., components that are useful for the natural organism, but not necessarily for the function of the desired application).

In conclusion, Benner continues to work toward his goal to develop a system that is self-sustaining and capable of evolving.

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BACTERIORHODOPSIN: A MODEL PROTON ION PUMP

> *We should take advantage of what was learned by biology, and take this knowledge and transfer it for our own uses.*
>
> –Janos Lanyi

Janos K. Lanyi, from the University of California, Irvine, studies the physiology, biochemistry, and molecular mechanisms of ion translocation across biological membranes. One system he has studied for many years is the light-driven electrogenic transmembrane proton ion pump in the cytoplasmic membrane of *Halobacterium salinarum*, called bacteriorhodopsin (BR), which he spoke about in detail during the workshop.

Lanyi provided an overview of how biological systems work and discussed key principles needed to construct useful energy collection

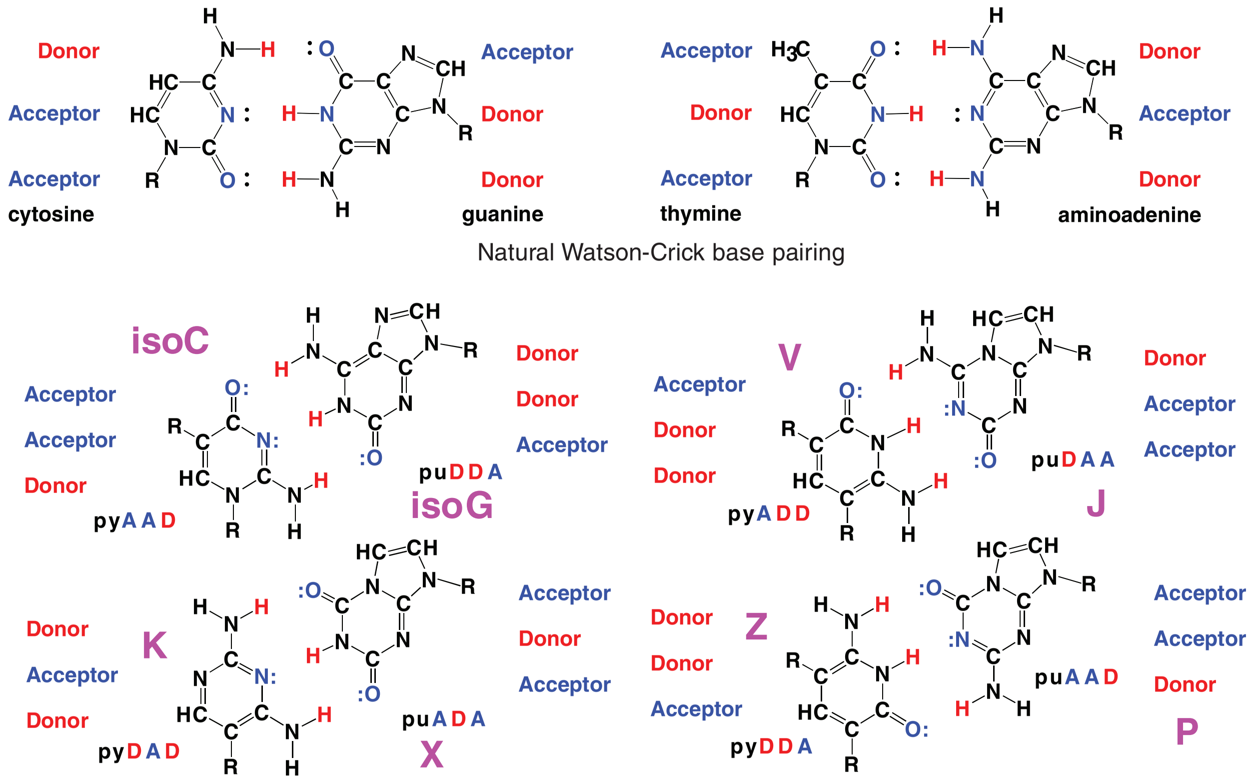

FIGURE 2-4 (Top) Natural Watson-Crick nucleic acid base pairs adenine (A), cysteine (C), guanine (G), and thymine (T); (Bottom) Benner's artificial orthogonal DNA base pairs K, X, ZP, V, J, iso-C, and iso-G, created by shuffling donors/acceptors.
SOURCE: Stephen Benner, presentation slide.

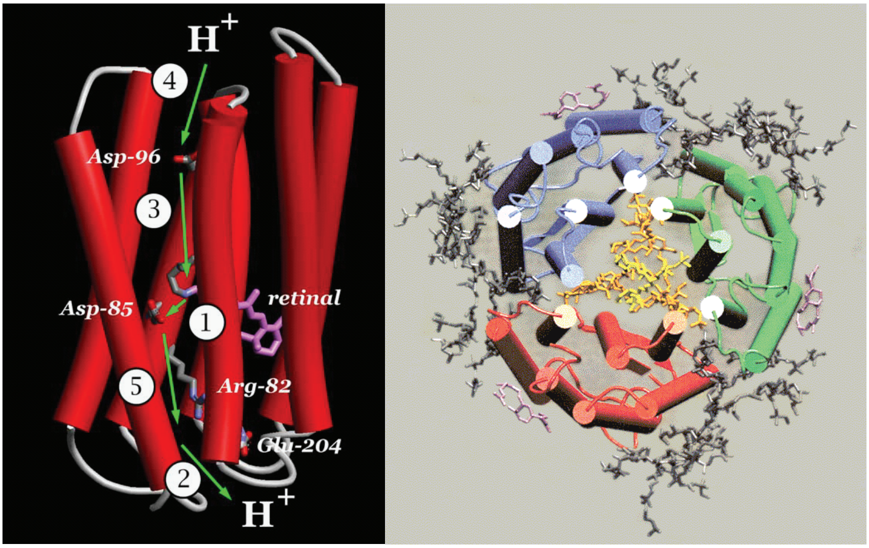

FIGURE 2-5 (Left) Ribbon diagram side view of bacteriorhodopsin showing movement of the proton at each step of the photocycle. (Right) Cartoon representation of top view of BR as it exists in nature, with three BR protein monomer units surrounded by lipid molecules. The three BR monomers are shown in blue, red, and green.
SOURCE: (Left) Adapted from Lanyi (2004). (Right) Baudry et al. (2001).

systems and/or biologically inspired technologies. He then focused on BR (Lanyi, 2004), which is a small membrane protein with a single type of subunit (homooligomer as depicted in Figure 2-5) that contains a single retinal chromophore. Lanyi and others study BR to discover new information about the mechanism of proton transport. BR is an ideal system to study, because of its simplicity compared with other kinds of proton pumps (Table 2-1).

As described by Lanyi, in a retinal-based ion pump, retinal isomerization after photon absorption initiates molecular rearrangements and results in ion uptake on one side of the membrane, translocation, and then release on the other side—the extracellular side of halophilic archaea. Then, again, another proton is taken up from the cytoplasmic side in a later reaction, which results in a net electrogenic translocation of a proton. The specific plumbing part of this pump is known, as is the proton part. Lanyi said that one of the important questions about the BR system is "How does the isomerization of the retinal chromophore in the reaction drive all the steps in this larger protein?" Lanyi said that the principle of this ion pump is that the strain in the distorted photoisomerized retinal

TABLE 2-1 Comparison of Several Types of Protein Pumps

Protein	Cofactor, Substrate, etc.	MW	Subunits
Mitochondrial cytochrome oxidase	Hemes, Cu, Fe	130,000	13
Mitochondrial cytochrome bc1	Hemes, FeS, ubiquinone	225,000	11
Mitochondrial ATPase	ATP/ADP	350,000	>20
Mammalian NaK pump	ATP/ADP	280,000	8
Mammalian Ca pump	ATP/ADP	290,000	2
Bacteriorhodopsin	Retinal	24,000	1

SOURCE: Janos Lanyi, presentation slide.

gradually relaxes as the binding site accommodates its changed shape. Inherently, the relaxation includes deprotonation of the Schiff base. The cascade of conformational changes that ensues allows the release of a proton at one surface and uptake at the other.

In conclusion, Lanyi said BR serves as an important and useful model for better understanding of the mechanism of photoactivity and proton transport in biological systems that could help in developing future energy systems.

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NOVEL MECHANISMS OF ANAEROBIC METHANE OXIDATION

> *If, in nature… a principle was discovered [three times], then it's a good sign that that is the solution to the problem.*
>
> –Rudolf Thauer

The research of Rudolf K. Thauer, Max Planck Institute for Terrestrial Microbiology, focuses on the biochemistry of methanogens—microorganisms that make methane and other microorganisms that oxidize methane to CO_2—primarily from a methane seep area in the Western Black Sea (Figure 2-6).

Thauer discussed anaerobic oxidation of methane with sulfate as an electron acceptor. This is of great interest because previously the anaerobic oxidation of methane was thought to be impossible in biological systems,

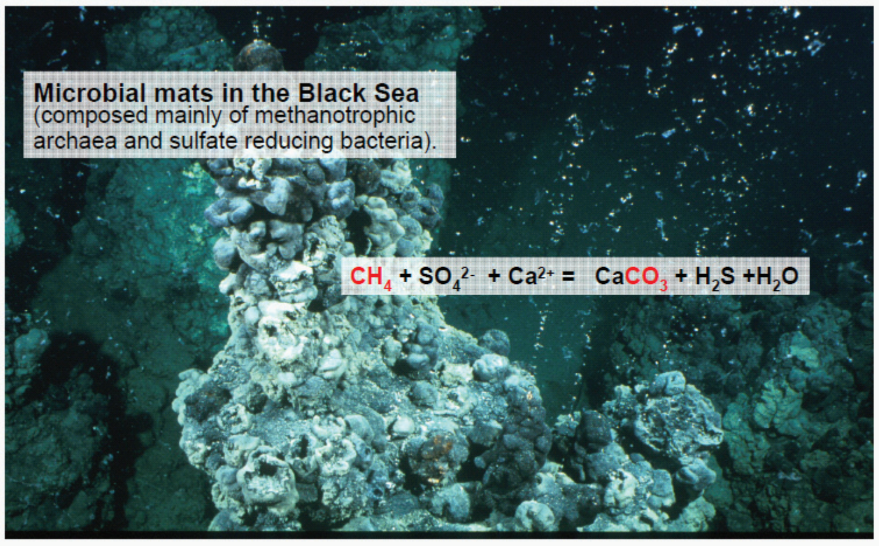

FIGURE 2-6 Microbial mats collected at cold methane seeps in the Black Sea carry out anaerobic oxidation of methane to carbon dioxide using sulfate as the electron acceptor. These mats, which predominantly consist of sulfate-reducing bacteria and archaea of the ANME-1 and ANME-2 types, contain large amounts of proteins very similar to methyl-coenzyme M reductase from methanogenic archaea.
SOURCE: Mayr et al. (2008).

because of the strength of the CH bond in methane (Thauer, 2010). To homolytically cleave the CH bond of methane, almost 440 kilojoules per mole of energy is needed. The only other bond that is on that order is the OH bond of water. One can attack methane with an OH radical, a scenario that happens in the atmosphere, but it appeared to be almost impossible to do that with a sulfur radical, or so it was thought. However, there was early evidence from Reeburgh in the 1970s that contradicts this. Now, Thauer said, there is no doubt that methane can be used by anaerobes to fuel their energy metabolism using either sulfate, Mn^{IV}, Fe^{III}, or nitrite as the terminal electron acceptor (Figure 2-7). However, the biochemistry of this process is still largely unknown.

Thauer studies microbes from the Western Black Sea that use a nickel catalyst to oxidize methane. It has now been shown that the nickel enzyme purified from a methanogen can catalyze the oxidation of methane to methyl-coenzyme M ($\Delta Go' = 30 \pm 10$ kJ/mol) with apparent K_m and V_{max} consistent with the values estimated for cultures catalyzing anaerobic oxidation of methane (AOM) with sulfate (Scheller et al., 2010). The crystal structure of the MCR homologs purified from Black Sea microbial mats

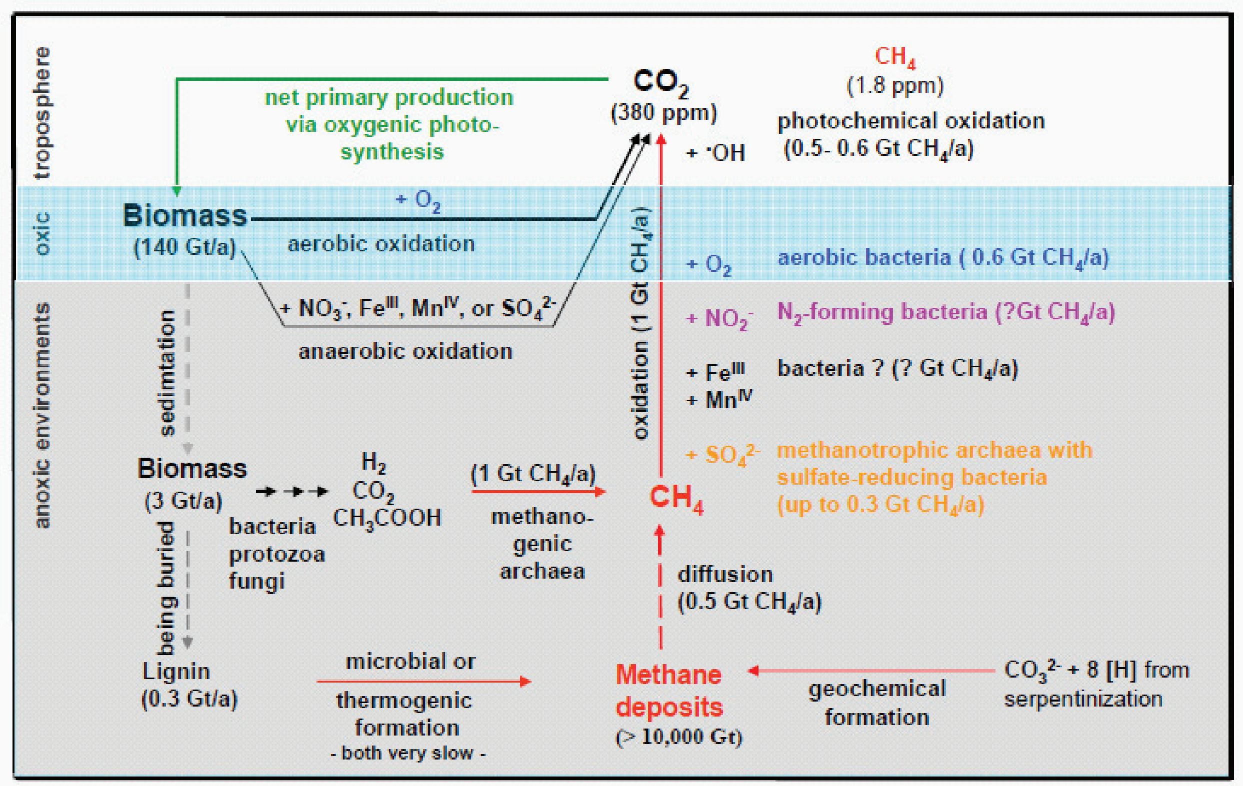

FIGURE 2-7 Schematic of the global methane cycle, which highlights the different microorganisms involved in catalyzing the anaerobic oxidation of methane with nitrite (magenta) and sulfate (orange).
SOURCE: Adapted from Thauer (2010).

catalyzing AOM with sulfate revealed that the enzyme from methanotrophic archaea uses the same coenzymes as methanogenic archaea, the best-studied system. It is therefore very likely that methane is oxidized to methyl-coenzyme M in methanotrophic archaea via the same mechanism as it is formed from methyl-coenzyme M in methanogenic archaea.

The key idea Thauer presented during his talk was that the mechanism used by microorganisms could inspire chemists to build catalysts that might be used to oxidize methane. This would be a major breakthrough because methane is the largest hydrocarbon source on Earth and storing it is problematic. However, it would also be beneficial if methane could be catalyzed to form methanol, because methanol can be easily stored and transported.

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SOLAR ENERGY HARVESTING IN THE EPICUTICLE OF THE ORIENTAL HORNET

> *There's a great importance today in science and industry for structures that can manipulate light. This area is extensively studied in insects and has inspired the development of many nanometric photonic structures.*
>
> –Marian Plotkin

Marian Plotkin, from the University of Singapore, discussed the possible ability of the oriental hornet to harvest solar energy (Plotkin et al., 2010). He began his presentation by describing the epicuticle of an oriental hornet (Figure 2-8), the external coating of its exoskeleton.

One of the interesting activities of the oriental hornet is its digging capability. Since the hornet builds its nest underground, it digs soil and small stones and then picks up the stones in its mouth and flies them a short distance to drop the stones from midair. It then returns to its nest to continue this process. The process is interesting because the digging activity is correlated with solar insulation, the intensity of solar radiation. The digging activity of oriental hornets is twice as much at noon as it is in the morning or in the evening. Studies conducted by Plotkin and others

FIGURE 2-8 Adult oriental hornet resting on silk caps.
SOURCE: Plotkin et al. (2010).

in the group show a statistically significant correlation between digging activity and UVB radiation.

Plotkin described the two types of cuticles, the brown and yellow cuticles (Figure 2-9), each having different pigments. The yellow cuticle contains a pigment believed to harvest solar energy for the oriental hornet. The brown cuticle is composed of the epicuticle, which is the outermost part of the cuticle, the exo-endocuticle, and the hypocuticle. Plotkin said that the size of the layers changes. The outermost layer is about 1 to 2 micrometers, while the deepest layer, the hypocuticle, is around 30 nanometers. The cuticle is brown because of a pigment called melanin, which is inside every layer. The yellow cuticle is a bit different from the brown cuticle. It also has the epicuticle, exo-endocuticle, and hypocutical layers. The layer sizes, however, are thinner than those of the brown cuticle. For example, there are about 15 layers at the exo-endocuticle, compared with 30 layers at the brown cuticle. One can see the yellow because it is contained in yellow pigment granules between the exo-endocuticle layer and the hypocuticle layer—xanthopterin.

Studies conducted by Plotkin and others in his group, found

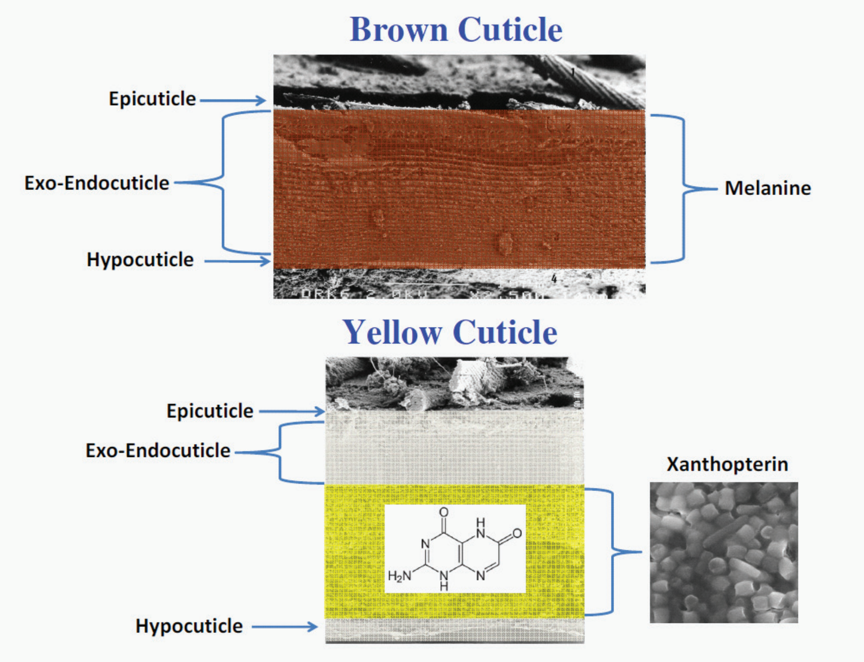

FIGURE 2-9 Cross sections of the brown and the yellow hornet cuticles.
SOURCE: Adapted from Plotkin et al. (2010).

- The oriental hornet cuticle exhibits antireflection and light-trapping properties. The measurements show that the complex layered structure increases the antireflective effectiveness. It is believed that the cuticular multilayered structure may introduce a gradual change in effective refractive index by varying the proportion of chitin nanorods within the protein matrix or by altering the angle of deposition of the nanorods.
    — The xanthopterin pigment acts as a light-harvesting molecule.
    — The oriental hornet correlates its digging activity with solar insulation.
    — The oriental hornet cuticle is a photonic structure capable of harvesting sunlight.
- The pigment xanthopterin within the cuticle transforms sunlight into electrical energy, which may serve the metabolic activity performed within the pigment layer, hence aiding in the digging activity. They concluded this by conducting an experiment using a dye-sensitized solar cell. They inserted the pigment xanthopterin inside the electrode, and as light was absorbed by the pig-

> ment and transformed to electricity, a current voltage measurement was taken. Plotkin said that this experiment indicates that xanthopterin is actually a light-harvesting molecule. It is believed by Plotkin that the electrical energy is converted into chemical energy to aid the hornet in its digging activity. However, he does not know how energy is transformed inside the oriental hornet; he believes the key to answering this question is understanding the mechanism at the molecular level.

Plotkin concluded by saying that there is a great need for structures that can manipulate light today in science and industry. This area is extensively studied in insects and has inspired the development of many nanometric photonic structures. Insects are able to construct durable, precise, and complex nanometric structures in their cuticle. Plotkin's main message was that the oriental hornet cuticle structure and its self-assembly processes during metamorphosis could serve as a model for improving the design of photonic structures.

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ENERGY PRODUCTION IN BIOFUEL CELLS

> *We have thousands of species, maybe millions of species of microbes…. They had to have invented wonderful machines to accomplish all the things that we want to do.*
>
> –Kenneth Nealson

Ken Nealson, University of Southern California and the J. Craig Venter Institute, specializes in research on microbial physiology and genomics, environmental microbiology, metagenomics of natural populations, and microbial fuel cells. At the workshop, Nealson focused on electromicrobiology, the *Shewanella* organism, and some of the lessons he and his group learned from studying microbial fuel cells.

"Electrons must flow," said Nealson. Electron flow is essential to life, and electron donors and electron acceptors define the thermodynamic range over which microbiology happens in our planet. The energy from electron flow is used to drive reactions of life. The geological environment supplies many oxidants and reductants that life has learned to utilize (Figure 2-10). For example, a methanogen does not have a lot of energy; in

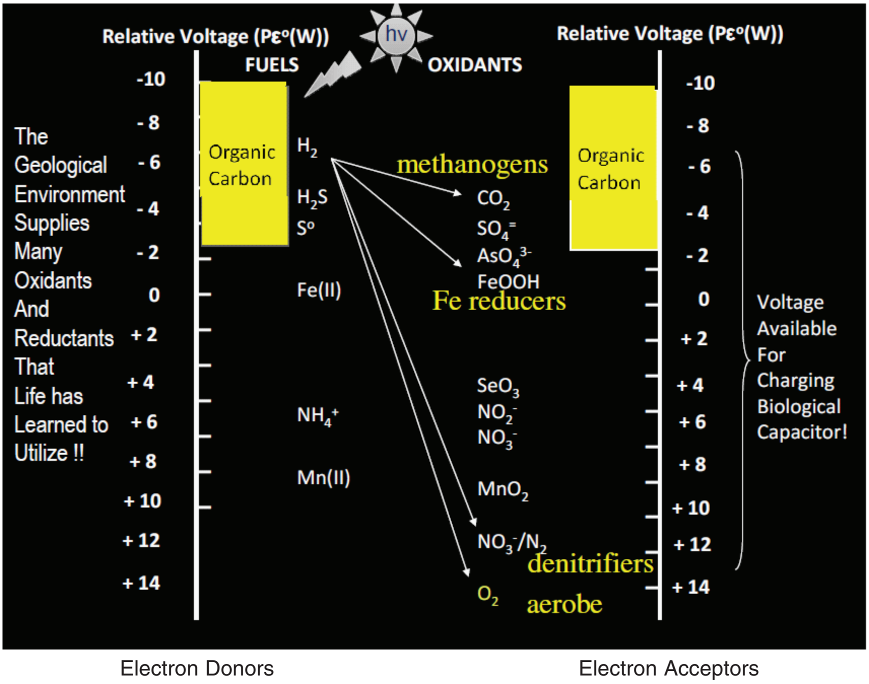

FIGURE 2-10 The relative voltage of electron donors (fuels, left) and electron acceptors (oxidants, right) show the thermodynamic foundation of microbial redox chemistry. A voltage is generated when a donor has a higher potential than an acceptor, provided all other conditions (pH, concentration of reactants, etc.) are acceptable. Life has evolved to use almost every one of the sets of redox couples. Eukaryotic life in general uses organic carbon as electron donor and oxygen as electron acceptor, whereas the prokaryotes (methanogens, FE reducers, and denitrifers) use many other acceptors, taking advantage of abundant and ubiquitous energy sources and oxidants available.
SOURCE: Ken Nealson, presentation slides.

fact it will consume energy to reproduce. However, iron reducers generate more energy than methanogens, but much less than aerobes, because oxygen is a favorite electron acceptor. Thus, the geology of Earth and the mineralogy of Earth and microbiology are strongly connected.

Nealson then spoke about a unique organism that he isolated about 24 years ago in the sediments of Lake Oneida, New York, which he named *Shewanella oneidensis* (Venkateswaran et al., 1999). Nealson said *Shewanella* “is a remarkable ‘bug’ that is capable of doing really remarkable environmental chemistry.” He said his laboratory found strange manganese and iron chemistry in a lake in New York that could not be explained by just chemistry, so they thought it must be due to a microbe capable of reducing iron and manganese solids, which led to the discovery of *Shewanella oneidensis*.

Nealson showed audience members a short video clip that was taken inside an anaerobic flat capillary. In the video, *Shewanella* was found to be swimming around and reducing tiny pieces of manganese oxide until all of this terminal electron acceptor was depleted. Under normal conditions, the manganese oxide is stable for about 10,000 years. However, working at low temperature, this biological catalyst was able to transform the very stable mineral about a million-fold faster than under normal weathering conditions.

Nealson described many important facts about *Shewanella* that his laboratory and others found through experiments over the years. The important facts include

- Genes for metal reduction are multiheme cytochromes.
- Cytochromes are on outer membranes (C. Myers, Medical College of Wisconsin).
- There are ~10,000 of each cytochrome per cell (Lower et al., 2007).
- Complex interactions exist between proteins (Ming Tien, Pennsylvania State University).
- Proteins are synthesized under anaerobic conditions (Daâd Saffarini, University of Wisconsin, Milwaukee).
- Some strains of *Shewanella* have duplicate genes (induced in biofilms, J. McLean, J. Craig Venter Institute).
- They are capable of electron transport to the anode of microbial fuel cells (Byung Hong Kim, Korean Institute of Science and Technology, and others).
- Under electron acceptor limitation, cells make electrically conductive nanowires (Y. Gorby, University of Southern California; Gorby et al., 2006; El-Naggar et al., 2010).
- Extracellular polymeric substance (EPS) with conductive metals (pyrite) also works.

Nealson and others sequenced about 20 different strains of *Shewanella*, and because they had the sequence, they made deletion mutants that were nonpolar in particular spots. They found by deleting virtually all the cytochromes in this microbe one at a time that there are three enzymes that are the main working parts for metal reduction. They are MtrA, MtrB, and MtrC (Mtr = metal reduction), as shown in the conceptual model for extracellular electron transfer in *Shewanella sp.* in Figure 2-11. They found that if the genes that produce these three enzymes are put into *Escherichia coli*, the bacteria will reduce iron and manganese. A key point Nealson

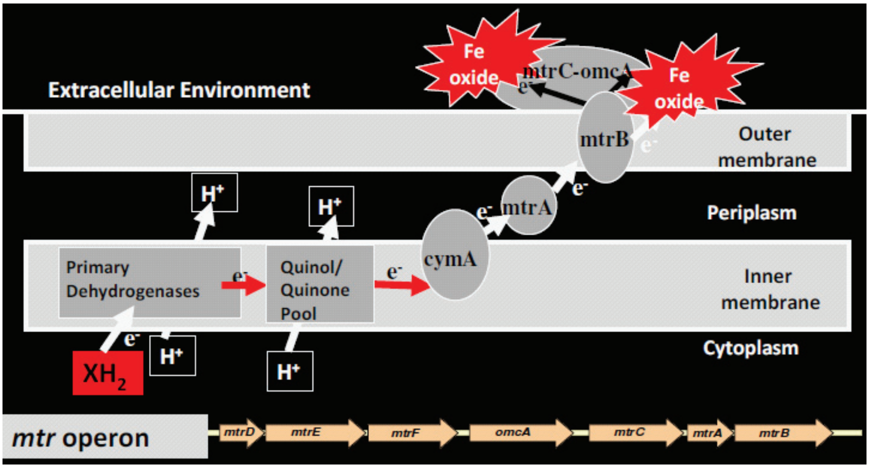

FIGURE 2-11 Conceptual model for extracellular electron transfer in *Shewanella sp*.
SOURCE: Ken Nealson, presentation slide.

made is that MtrA, B, and C are all decaheme cytochromes. On the outside of the cell is an MtrC complex. Spanning the pariplasm is MtrA, which is also a multiheme cytochrome.

Nealson then spoke about the modes of extracellular transfer, highlighting three types:

1. Direct transfer using multiheme cytochromes located on the cell exterior,
2. Biogenic environmental soluble mediators, and
3. Bacterial nanowires (or conductive EPS).

Lastly, Nealson spoke about how his laboratory has worked on developing *Shewanella*-based microbial fuel cells, which produce electricity from the metabolism of the microbe (e.g., He et al., 2009). Initially, his group developed fuel cells to take advantage of the electron transfer properties of the bacteria in an anaerobic environment coupled to an abiotic electron acceptor on the other side of the cell. More recently, Nealson and coworkers found the *Shewanella* metabolism could be engineered to be both an electron donor in an anerobic environment at the anode and an electron acceptor in an aerobic environment at the cathode of the fuel cell, what he described as “stretching out the microbes’ metabolism and putting it to work for humans.” He showed preliminary images of the fuel cell being used to purify polluted water, which indicate the possibility of using these microbial fuel cells for a low-cost (low-energy) solution for wastewater treatment, especially in the developing world.

In summary, Nealson highlighted the importance of exploring unique biological systems such as *Shewanella*, which he believes have a lot to teach humans about harvesting energy from unconventional sources.

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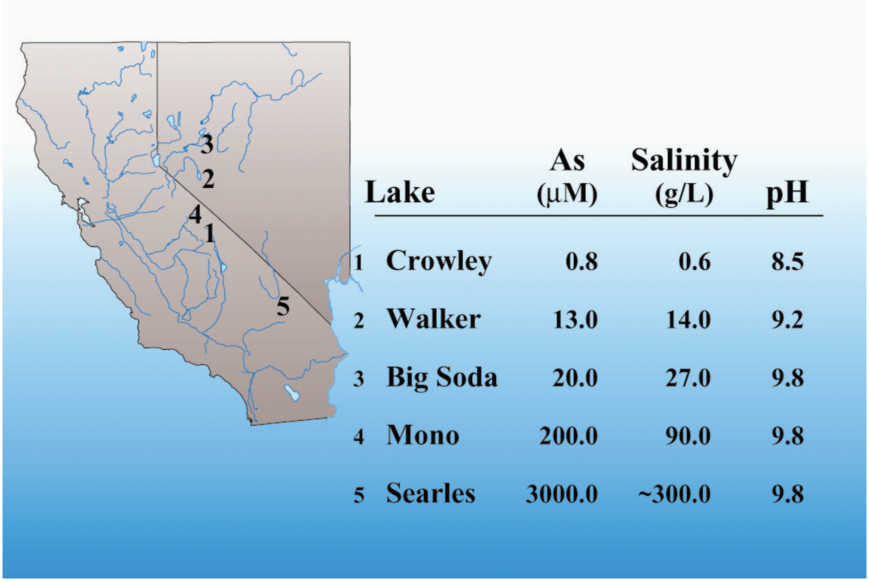
MICROBES AND THE FOUR BASIC STRATEGIES FOR LIFE ON EARTH

> *There are these specific solutions [in nature] and yet an inherent flexibility to be able to adapt as those things change.*
>
> –Felisa Wolfe-Simon

Felisa Wolfe-Simon, a NASA Astrobiology Research Fellow, highlighted the inherent flexibility of biology. She said life on Earth is metabolically diverse and yet maintains a biochemical unity, and the basis for all life starts with chemical underpinnings. This chemical potential manifests in four metabolic strategies used by life on Earth today, all of which most likely evolved in the distant past; they are photoautotrophic, photoheterotrophic, chemoautotrophic, and chemoheterotrophic. However, a single metabolism is not linked to a unique microbe. Wolfe-Simon said biological systems, which have a 3.5-billion-year history on Earth, are useful models to learn something about energy, because they have had a long time to evolve and learn unique and varied energy solutions. While there are some very specific energy solutions that have evolved in biological systems, there is also an inherent flexibility in those solutions that has allowed the systems to adapt overtime to a changing environment.

Wolfe-Simon discussed two of the projects she is working on. One of the projects is exploring photosynthesis in cyanobacteria that do not make oxygen (anoxygenic), and how this system can help understand the flexibility of "light-driven energy capture" (Johnston et al., 2009; Chauhan et al., 2011). Cyanobacteria are capable of facultative anoxygenic photosynthesis using a variety of electron donors in addition to water.

The other project Wolfe-Simon spoke about is the recently published research on a microbe, GFAJ-1 (found in high-arsenic soda lakes in California and Nevada, Figure 2-12), that appears to sustain its growth using arsenic instead of phosphorus, for example, in DNA (Wolfe-Simon et al., 2011). This result is important because all life is known to require six critical elements—carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulfur—and these elements are used to make important molecules such as DNA, RNA, proteins, and lipids. However, GFAJ-1 appears to be able to use arsenic when phosphorus is not available. Wolfe-Simon and her coworkers found that arsenic is present in the microbe wherever phosphorus is.



Lake	As (µM)	Salinity (g/L)	pH
1 Crowley	0.8	0.6	8.5
2 Walker	13.0	14.0	9.2
3 Big Soda	20.0	27.0	9.8
4 Mono	200.0	90.0	9.8
5 Searles	3000.0	~300.0	9.8

FIGURE 2-12 High-arsenic soda lakes found in the western United States, where arsenic-tolerant cyanobaceria GFAJ-1 exist.
SOURCE: Felisa Wolfe-Simon, presentation slide.

At the time of the workshop, the publication of this work had been met with skepticism and criticism in the scientific research community, which Wolfe-Simon discussed. Since then, much of debate has been captured on the editorial pages of *Science* (Pennisi, 2011).

Wolfe-Simon concluded by saying that the next steps in learning more about the role of arsenic in the GFAJ-1 bacteria would include synchrotron studies, in-depth physiological characterization, and genomic sequencing. In addition, cultures of GFAJ-1 have been made available to other groups to study independently.

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DNA NANOTECHNOLOGY

> *Many biological systems actually work isothermally; that means you are not heating things up to vast temperatures and using a lot of energy there.*
>
> –Nadrian C. Seeman

Nadrian C. Seeman, from New York University, discussed his work on using DNA's chemical information for bottom-up nanoscale control of the structure of matter and taking natural material and making an unnatural object from it. More biomimetic than bioinspired, Seeman described how the chemical information contained in DNA can be used to direct methods of organizing matter and making nanoscale mechanical devices such as assembly lines.

Seeman opened his remarks by reviewing what DNA is—the genetic material of people, plants, and animals. Seeman focused the rest of his presentation on highlighting DNA, the molecule, and what can be done with DNA outside of its biological context.

DNA, in its natural form, is linear topologically because it is unbranched. In its natural state, Seeman said it is not useful for construction of nanoscale mechanical devices, but it does contain genetic information making it an excellent tool for biomimetic synthesis. To produce

FIGURE 2-13 Reciprocal exhange of DNA, an idea Seeman "stole" from nature to create new DNA motifs. The blue and red lines represent two different sequences of DNA. After reciprocal exchange, the blue strand and red strand bedcome red and blue strands.
SOURCE: Nadrian Seeman, presentation slides.

structures more diverse than the natural DNA double helix, Seeman says he stole the idea of DNA and reciprocal exchange from nature (Seeman, 2001). He added "some people use the term bioinspired; I use the term biokleptic." Figure 2-13 shows a simplistic illustration of reciprocal exchange, where two different DNA sequences combine to produce new structures.

Branched species can then be connected to one another using the same interactions that genetic engineers use to produce constructs, that is, cohesion by molecules tailed in complementary single-stranded overhangs known as "sticky ends." Such sticky-ended cohesion is used to produce N-connected objects, crystalline lattices, and complex nanomechanical devices such as assembly lines.

The objectives and applications in Seeman's laboratory include architectural control and scaffolding, producing nanomechanical devices such as nanorobotics and nanofabrication, and self-replicable systems (Gu et al., 2010). Seeman said his lab focuses on DNA because "nucleic acid sequences can be programmed and synthesized, leading to information-based structural, dynamic, and catalytic chemistry."

As illustrated in Figure 2-14, the intellectual goal of structural DNA nanotechnology is to control the structure of matter in three dimensions (3D) to the highest extent possible and to understand the connection between the molecular and macroscopic scales (Zheng et al., 2009). Seeman said that the ability to control matter in this way has the potential to open new functionality of relevance to energy and other applications. For example, as discussed during the workshop, the 3D lattice structures Seeman created could have applications for separations or storage of small proteins or other molecules.

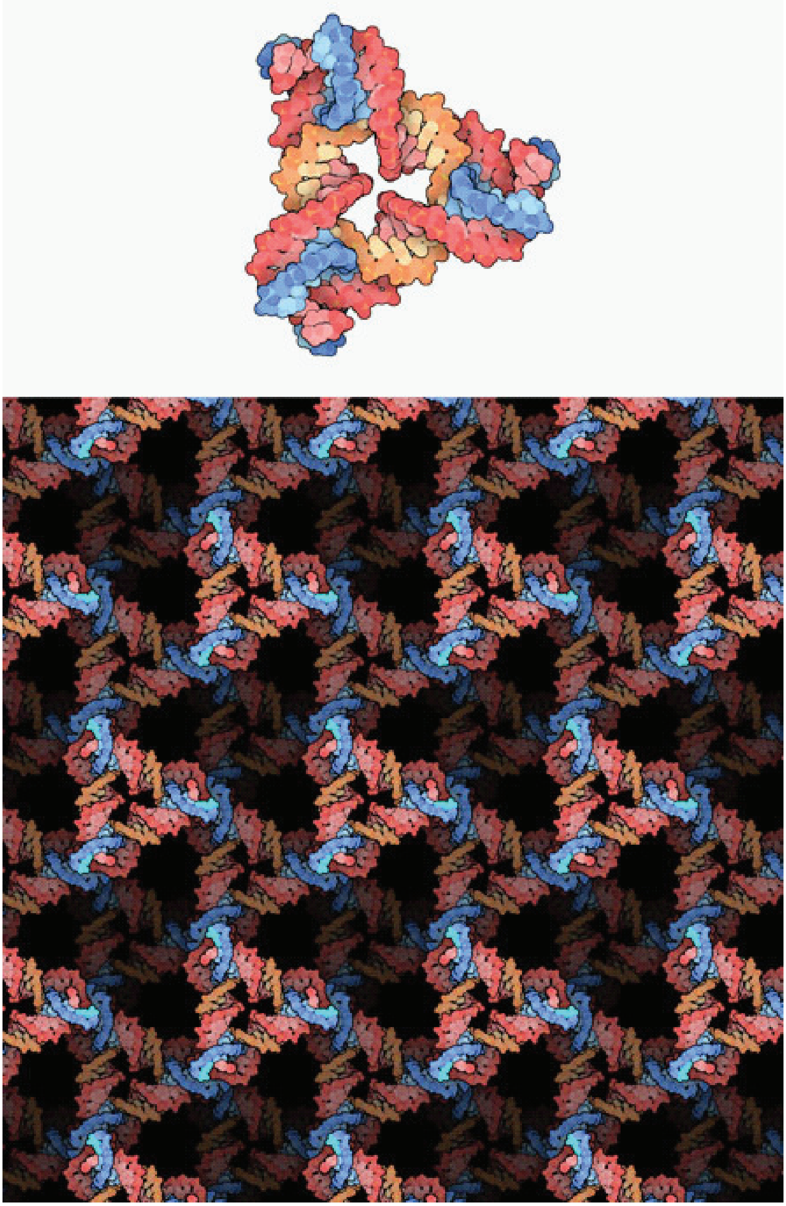

FIGURE 2-14 Illustrations of DNA structures. (Top) Branched DNA unit, with blue representing the connecting sticky ends. (Bottom) Three-dimensional array of engineered DNA units created by Seeman and coworkers.
SOURCE: Illustrations by David Goodsell, based on the RCSB Protein Data Bank entry 3gbi. Used by permission.

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3

Summary of Key Breakout Discussion Topics

Workshop participants met in three different breakout discussion groups during the course of the workshop. Each topical session of the workshop included a breakout discussion and a report-back time. The smaller size groups in the breakout sessions allowed for more in-depth and interactive discussion between workshop participants. The composition of the discussion groups was multidisciplinary and was meant to provide feedback to the larger group on key issues raised or important information provided by the guest speakers; research opportunities, especially for interdisciplinary collaborations; and resource and educational needs to support long-term advances. The key topics of discussion that came out of the breakout sessions (as reported back to all workshop participants) are described in detail in this chapter, and they are organized into the following general areas:

- Defining “bioinspired”;
- Microbial diversity and setting priorities;
- Research and collaborative models;
- Interdisciplinary education, training, and outreach;
- Microbial nanowires and fuel cells;
- Synthetic biology; and
- The big picture.

The key topics discussed in the following sections were suggested by breakout group participants, who were not vetted for conflicts of interest or biases, and they therefore do not represent conclusions or recommendations of the workshop organizing committee or the National Research Council.

DEFINING "BIOINPIRED"

Given the diverse disciplinary backgrounds of the workshop participants, a key question raised was "what does bioinspired mean?"

Mimicry Versus Inspiration

The term "bioinspired" is often used interchangeably with the term "biomimicry." However, biomimicry really means to copy or recreate natural systems, whereas bioinspired is about learning from nature to make something new. One participant explained that bioinspired really means to deeply understand the biological system being studied. Only when the biology is understood at the most fundamental level will it be possible to redesign it and create a better system. Artificial systems are desired over natural systems because biological systems often contain extra "baggage" (i.e., components that are useful for the organism, but not necessarily for the desired application function). Although there are advantages to natural systems—for example, proteins can be excellent catalysts in the form of enzymes—they tend to have limitations. In the cell, enzymes need to compete with many other substrates. Cells spend a lot of time and energy trying to engineer specificity, which may or may not be needed for energy applications. In most cases, much of the natural structure is probably not needed in artificial systems. The specificity may not be needed where the enzyme can be artificially inundated with a large amount of substrate.

One example discussed by some workshop participants is nitrogenase, which is a very important nitrogen-fixing enzyme related to both agriculture and energy. James Liao noted that about 5 percent of the energy used in the world is spent in the synthetic nitrogen-fixing Haber process (catalytic reaction of hydrogen and nitrogen to produce ammonia) (Smith, 2002). Unfortunately, nitrogenase cannot currently serve as an alternative method for large-scale nitrogen fixation to compete with the Haber process. The enzyme utilizes a very complicated process, involving 16 adenosine triphosphates and many electrons. Despite all the efforts to study nitrogenases for many decades, the mechanism of the enzyme is still unknown. Liao said that if someone could understand nitrogenase better or design an artificial enzyme based on or inspired by nitrogenase, it could be a major contribution to reducing the amount of energy used in the world for nitrogen fixation.

Many participants considered biomimicry to be a nearly impossible goal to achieve. For example, trying to mimic the relatively simple bacteriorhodopsin photosystem—or even just its photosynthetic center—seemed virtually impossible. There would be a long way to go to reach the point of being able to recreate or mimic even such a simple system.

Complete Understanding of Biological Systems

Another group reported back that a complete understanding of biological systems is needed to inform the development of synthetic systems. Janet Westpheling quoted Richard Feynman, who once said, "What I cannot create, I do not understand" (Feynman, 1988). However, it was not clear to many participants that successful creation of synthetic systems informed by design principles from nature will be significantly better than what nature itself has evolved. For example, Penelope Boston noted that a great deal of innovation has gone forward without that deep understanding and that, in fact, the very act of innovating and engineering design solutions has actually pushed the science in some fields. Tom Moore added that although a deep and complete understanding is the goal, it is not a prerequisite for moving forward on new ideas.

Chemically Inspired Microbiology

A participant suggested that the group should also talk about "chemically inspired microbiology"—that is, using chemical knowledge to drive biological exploration. Participants highlighted two examples of biological discoveries made because of chemical insights. Karl Stetter,[1] who often worked with biogeochemists, used his chemical insight to isolate novel microorganisms. Georg Fuchs[2] similarly used his chemical knowledge in discovering novel autotrophic CO_2-fixation pathways. In Fuchs' case, the genomes of the organisms found were already known, but people did not know to look for the chemical pathways.

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[1] For more information, see http://www.biologie.uni-regensburg.de/Mikrobio/Stetter/ (accessed September 1, 2011).

[2] For more information, see http://portal.uni-freiburg.de/ag-fuchs (accessed December 21, 2011).

MICROBIAL DIVERSITY AND SETTING PRIORITIES

Some participants noted that many of the speaker talks illustrated how biology provides a diversity of energy solutions; however, it is not always clear how to apply that diversity to meet human needs. Sometimes the diversity is so great that it is almost paralyzing for the research community. Since it is possible to culture about 1 percent of the microbial biota on Earth (Pace, 2009), and "only about 10 percent of the kinds of organisms on Earth are known" (Wilson, 2006), there is enormous biological diversity that is untapped. However, some participants questioned whether there should be much investment in culturing these organisms. Prioritizing the approaches for bioinspired energy may be needed. Questions to be addressed include

- What is most important to study in biology, and how can that be determined?
- Which aspects should be applied to the energy problems?
- What exactly does "bioenergy" mean?
  — Is it biomass? Is it solar? Or is it a combination of many different forms of energy transformation using biological components?

Westpheling explained that right now it is hard to design a path because the "there" is unknown. She said that one of the challenges is defining the destination, before the science and technology can be developed.

Thus, many participants said that there is a need to carefully identify the really important energy transformation problems and make sure that there is a potential biological solution to the challenges being addressed. For example, cost efficiency might be considered, or perhaps carbon-carbon bond formation and its importance to energy storage.

Culturing Bacteria

Penelope Boston pointed out that culturing brings organisms into a state where they can actually be studied. She said that it is useful to know about all the genomic biodiversity, but the microbes can really only be studied and manipulated if they are cultured. Although it may not be necessary to culture everything, she said that it is necessary to culture the right ones that are of interest as model systems and have genetic talents that can be manifested for these uses. Westpheling agreed and added that because approximately 50 percent of predicted genes are understood, there is much progress that needs to be made.

Conserved Microbe Functions

Some participants noted that one way of prioritizing biological models systems may be to consider the unknown and conserved hypothetical proteins that are available (Galperin and Koonin, 2004). The conserved hypothetical proteins are found across a wide array of organisms. Additional culturing may aid in this understanding. Understanding the biochemistry of microbe functions is also critically important. Some participants noted that it is not enough to know about the genomic sequence or array of proteins expressed—the chemistry of those proteins and what they can do also needs to be understood.

Biological Dark Matter

The related topic of genome annotation (attaching biological information to gene sequences) (Stein, 2001), or what Ken Nealson referred to as the "biological dark matter" of the genome, was also discussed by some workshop participants. Nealson said that there are many problems in annotation. It can lead people to believe in false assumptions, because of incorrect annotations. For example, as Janos Lanyi explained, changing a single amino acid residue in bacteriorhodopsin transforms it from a chloride pump to a proton pump. This would have never been discovered with annotations, because the genome sequence would not have provided such an insight. Another example Nealson gave was for related Crp-FNR DNA binding proteins,[3] which he said are found in both *Shewanella* and *Escherichia coli*, but regulate in *Shewanella* opposite to what they do in *E. coli*. In *Shewanella*, FNR regulates sugar metabolism and Crp regulates anaerobic/aerobic response, whereas in *E. coli* the two are reversed. He said that even when a genome sequence is properly annotated for one organism, the annotation may not apply to the same genomic sequence in a different organism. This presents a big problem to be addressed.

Living Systems Baggage

Doug Ray mentioned that it has been found that organisms in extreme environments may have less biological "baggage." For example, *E. coli* is considered to have more excess components than most other microbial organisms. He said that before concluding the nonutility of organisms because of baggage, it may be necessary to think a little more broadly about the organisms that are present and available.

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[3] Crp-FNR = Cyclic AMP (cAMP) receptor protein (Crp)/fumarate nitrate reductase (FNR) regulator. For more information, see Körner et al. (2003).

Following Environmental Clues

Julie Maupin-Furlow pointed out that the work of Rolf Thauer, and an understanding of the redox environment in nature, can drive an approach to isolate organisms. She said microbiologists often do not take such an approach and biological information is missed, because some researchers address the wrong problem. As Thauer mentioned, many researchers presumed the anaerobic oxidation of methane (AOM) to methanol was not occurring because the CH bond in methane is one of the strongest aliphatic CH bonds. Fortunately, Thauer and other biogeochemists probed further and challenged the dogma of the time, and showed that AOM was occurring. This highlights the importance of bringing in people from one field to challenge those in other fields. Maupin-Furlow said some might call what Thauer did “microbiology myth busters.”

Some participants also talked about looking at energy-limited systems for inspiration, such as methanogens. Such systems utilize very little energy. The efficiency of enzymes that are catalyzing reactions in such systems are very different when compared with the enzymes involved in photosynthesis, in which there is typically excess energy, such as the “RuBisCo” (ribulose bisphosphate carboxylase/oxygenase) enzyme system in plants.

Reversible Processes

Some participants noted that it is a thermodynamic paradigm that says a reversible process is the most efficient process. Some mainstream biological processes are reversible, including those that generate proton-motive force. However, some key enzymes involved in energy transformations are irreversible, including redox enzymes. The opportunity for rational design of redox cofactors, particularly with respect to reversibility, might offer ways to understand natural systems better and perhaps to devise systems that could meet the energy requirements of humans.

Matching the Solar Spectrum

Tom Moore brought up the solar spectrum and photosynthesis. He said photosynthetic chemical work is not well matched to the solar spectrum. The photons in the blue region of the spectrum are much more energy-rich than those in the red region of the spectrum, but photosynthesis degrades all the photons to the red region of the spectrum. Human technology offers opportunities to incorporate some of the multijunction features into biological systems, although this has not been done yet.

Nutrient Cycles

James Liao pointed out that making the nitrogen cycle more efficient has also been largely ignored. A lot of focus has been on understanding the carbon cycle, but the nitrogen cycle is equally important. It consumes energy and contributes to the greenhouse effect dramatically.

Liao also said there are at least four different CO_2-fixation cycles found in biological systems (Thauer, 2007). He said that those are important cycles, but the challenge for scientists is to do better. So far, to his knowledge, no one has even proposed an artificial working cycle that fixes CO_2 in a biological setting.

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RESEARCH AND COLLABORATIVE MODELS

Workshop participants discussed what is needed to support research in bioinspired energy.

Importance of Discovery Science

Julie Maupin-Furlow said that a lot of time in one of the breakout sessions was spent discussing task-oriented versus discovery-based and fundamental research. Many in the group felt that it is important to balance discovery-based findings with hypothesis-driven projects. She stressed the need for balance.

Doug Ray also commented that developing and testing a hypothesis is important, but it needs to be done in conjunction with discovery or exploratory science. There is some sense that it is not possible to get funding for discovery science. It is important to balance the two—not to the exclusion of each other.

Janet Westpheling pointed out that once a promising biological system is discovered, there is then a need for public and private research institutes to provide a model for how to transition from academic discovery to commercial development. There seems to be a gap between basic science and the application of the basic science that needs to be filled. Some participants suggested the national laboratories as one possibility. Other models discussed included the following:

IBM model. A participant who worked with IBM Research said one problem they faced was incorporating findings from the research laboratory into products. One successful approach was to have the people who were designing the technology to be on the implementation team. However, that can require an entirely new personnel and communication system.

Pharmaceutical company model. Janet Westpheling said that the model followed in large-scale pharmaceutical companies can also be effective. She worked for a pharmaceutical company and was involved in developing products from biological systems. They would have product meetings with different teams focused on everything from the research development of the organism that made the product, to the people who bought the raw materials that went into the fermentation, to the engineers, to the marketing people. No one would conduct an experiment that could not be carried out in practice in an industrial setting. Another participant added that these transformations in the pharmaceutical companies started in the 1980s and took time to show results. There had to be an effective collaborative team.

Bell Labs model. Greg Petsko discussed the Bell Labs model, where some people did basic research and other people did applied research, all in the same building. A participant mentioned how Steve Chu, the Secretary of Energy, refers to the model in almost every speech he gives. Chu worked at Bell Labs for 6 years, and he often talks about how he wants to use the model for Department of Energy (DOE) labs (Chu, 2009; Morford, 2009). However, some questioned whether this model can still work in contemporary times. It may not be possible to hire the same type of talented people and give them freedom to experiment. Now, results are expected in a much shorter time frame—on the order of months, not years.

DOE bioenergy centers. Westpheling argued that it is not always necessary to be in the same building for effective collaboration to occur. She is now part of the DOE-funded Bioenergy Science Center that is led by Oak Ridge National Laboratory. It is a virtual center that brings together people from all over the country. She said that it is not always feasible to bring together the equipment and the expertise in one place, or to get people to move to the same city to be involved in the same work. This is another model of reaching out to the larger community that has been shown to work.

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INTERDISCIPLINARY EDUCATION, TRAINING, AND OUTREACH

Given the interdisciplinary nature of bioinspired research, many participants spent a lot of time talking about interdisciplinary training, or simply approaching their research in an interdisciplinary way. Many people in the group felt that it is most important to have the core curriculum intact and then, once there is a biological problem or a problem that needs to be solved, drive interdisciplinary work.

Janet Westpheling added that while interdisciplinary training of scientists is critical, there is concern about how it can be done without "watering down" the individual disciplines.

Effective Communication

Many participants noted that interdisciplinary education, training, and outreach are needed so that efforts in bioinspired energy can be effectively communicated. Energy has been compared to the space program. Everyone supported the idea of the space program, because putting a man on the Moon was an exciting proposal. But that kind of enthusiasm is currently not there for energy—even though energy might be an equally important or even more important goal as going to the Moon. Outreach to the general public is an important aspect of effective communication.

Because the participants came from many different disciplines, there was a lot of discussion about disciplinary language differences, and how to make that communication more efficient. Someone suggested that perhaps a workshop for workshops is needed—to explore how groups can learn each other's disciplinary language to enable more efficient communication. Without making some improvements in communication, scientists may be unable to effectively speak with one another about their work.

One participant commented on the need to effectively communicate about energy research to the general public and to the funding agencies. He said the message should be that studying biological energy systems is the most fundamental and basic research that can be done on the planet. It is the findings and rational design principles based on physics and chemistry that are going to underpin synthetic biology—if nine billion people are going to be supported on the planet, some fundamentally new approaches in energy are needed.

Cross-Disciplinary Research and Training

Ken Nealson commented on cross-disciplinary training efforts, especially the positive impact he has witnessed in interdisciplinary summer courses. He said there is a huge transformation that happens to students during these summer courses. If the course is taught effectively, the students are exposed to many different disciplines. He now teaches a course in geobiology and previously taught one in the past on planetary biology, which involved isotope chemists, organic chemists, and microbiologists. In the course, he lectured and conducted labs with the students. He saw interdisciplinary workers emerge from these courses; they did not have to take a course in interdisciplinary science—they had to see how exciting it was. He said this was an effective way for funding agencies to invest in the future. It was a 10-year time period (largely during the 1980s) (Nealson and Nealson, 1993) when the National Aeronautics and Space Administration (NASA) funded the Planetary Biology and Microbial Ecology program.

Nealson said that many current rising stars in the field (including speaker Felisa Wolfe-Simon) attended those summer courses. He encouraged funding agencies to continue to support these successful interdisciplinary courses, which offer a great opportunity for the next generation. Wolfe-Simon added that the United States also needs centers that foster interdisciplinary work—especially for the up-and-coming researchers, who need to be supported and given freedom to explore. She added that while she is a fan of new media, there are significant advantages to being able to walk down the hallway or the next building to talk about research with a colleague in astronomy or engineering. Skype does not provide that type of interaction. She does not know what might be the mechanism for creating the centers, but she said this country needs a place where an early-career scientist has some freedom to do science, because research faculty members are often overwhelmed by large teaching loads, committee memberships, or other responsibilities.

Many participants discussed how it may take a combination of activities, centers, summer courses, and people visiting different labs, with funding from multiple agencies to support the interdisciplinary research and educational needs.

Current Opportunities

Robert Stack described current DOE support for the microbial ecology summer school at Woods Hole.[4] It is a 6-week summer class, which does exactly what Nealson and Wolfe-Simon discussed. The class is funded jointly by the DOE Biological and Environmental Research program and the Basic Energy Sciencesprogram, the NASA Astrobiology program, and the National Science Foundation. However, he said that it is a very expensive class, given that only 20 students take the course each year. There is a principal investigator for the class that rotates every 3 to 5 years, and the instructors change.

Stack said DOE also often puts together workshops to achieve similar goals. They try hard to build interdisciplinary working relationships by holding annual or biannual contractor meetings. For example, everyone funded by his program in physical biosciences meets every 2 years. It includes everyone from chemists, to x-ray crystallographers, electron paramagnetic resonance spectroscopists, molecular biologists, and microbial ecologists. DOE also provides seed money to people who have great new ideas.

The group discussed the value of this workshop and the need for more in which a variety of kinds of expertise are brought together to address a common theme.

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SYNTHETIC BIOLOGY

Many participants talked about how to use the molecules that are discovered in biological systems, such as the photoactive chromophore of bacteriorhodopsin in harvesting energy. Bacteriorhodopsin is a very

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[4] Microbial Diversity Summer Course at Woods Hole [online]. Available: http://www.mbl.edu/education/courses/summer/course_micro_div.html and http://courses.mbl.edu/microbialdiversity/ (accessed Sept. 22, 2011).

successful system for converting photoenergy to proton-motive force. However, it is not clear how this proton-motive force can be used in an energy production system on a large scale. Some participants asked what might be done to understand this system better. For example, are there synthetic analogs of the transformation in retinal? Is there any way to simplify the system so that protons can be pumped on a large scale? Instead of limiting it to the cell membrane, can the system be designed on a large enough scale to harness this energy?

Rational Design of Proteins

As Les Dutton indicated, it is possible through rational design to think about creating synthetic electron transfer proteins and components of future synthetic biology systems. Dutton also mentioned that it is important to focus on the rational design of proton transfer systems—proton pumps or proton-transfer-linked transducers that can run in two directions. In one direction, these proton-transfer systems would pump protons against a proton electrochemical gradient, and in the other direction, they would do some work, chemical or mechanical, by transducing protons back across the system. Tom Moore said that, right now, no one really has an idea of how to rationally design a proton pump. He pointed out that it is important to remember that biology never operates without the combination of proton-motive force and electromotive force.

However, the progress on understanding the two systems is uneven. A lot is understood about the fundamentals of electron transfer systems, but not as much is known about proton transfer. Moore said protons offer an incredibly rich research area, because protons are, in a sense, between classical and quantum mechanical particles. Sometimes their motion is not limited by mass transport considerations. Proton wires exist and, under short distances, protons can tunnel. Thus, he said that it is hoped that the environment for research in proton-motive force, particularly synthetic and artificial systems to generate proton-motive force and then couple that proton-motive force to either chemical or mechanical work, will increase in the future.

There was some discussion among participants about defining synthetic biology. Westpheling said that she thought the group meant it as the synthesis of microbial functions. Tom Moore added that he thinks it is an unlimited definition, because right now the field is open and no one knows where synthetic biology can lead. He said that if someone asked Bardeen to define the transistor in 1948, he probably would have given a similarly broad answer regarding semiconductor physics. Looking ahead, they knew they had to go somewhere that vacuum tubes could not take them, but it is worth noting that they did not have a path. They did not know where they were going. They just knew that they could not go there with vacuum tubes. The way they addressed that issue was to hire bright people and give them the freedom to explore. Moore said that it was the type of focused-up/diffused-down management and leadership that needs to be seen more.

There was a discussion about what the role of synthetic biology will be in developing bioinspired systems going forward. Many in the group believe that it is probably a long way to go from de novo synthesis of a biological system to a useful application. Modifying known biological systems is probably a more productive approach in the short term.

MICROBIAL NANOWIRES AND FUEL CELLS

Ken Nealson's talk about bacterial nanowires in *Shewanella* sparked a lot of discussion among participants about alternative electron transfer architectures, including the microbial synthesized nanowires (Gorby et al., 2006). There was a lot of interest in the ubiquity, or not, of nanowires and electrically conductive extracellular material in nature and what that might mean, how one might use that for different purposes.

Moore commented that he thinks this is one of the most fascinating things that has been discovered in the last 10-15 years—how living organisms use the nanowires to remove electrons from the system. He said, "In removing electrons from their cells, they in a sense feed themselves reduced carbon, electron carriers, that then are taken up in nutrition." The bacteria Nealson discussed in his presentation do the same thing. They take in an external source of reducing power of low-potential electrons and get rid of them, under conditions where other electron acceptors are not available. They move the electrons completely out of their cells through the nanowires. Moore noted that in order to keep charge balance, the bacteria also have to get rid of protons.

Microbial Nanowires

Les Dutton talked about how some researchers have incorporated the nanowires into artificial liposomal membranes and have studied electron transport within these systems. John Golbeck added that the idea of using long-distance microbial nanowires to connect one cell to another has great potential. He said that, right now, one of the problems in generating solar biofuels, such as making hydrogen, is that the electrons also need to come from water, which means that there is an oxygenic environment. The approach is to reduce protons with hydrogenase in an oxygenic environment, but that is not going to be possible with the current approach of using a single cell.

The wire enables a new approach, because now space and time are separated. For example, there could be an oxygenic environment in one cell connected with a wire and an anaerobic environment in another cell, completely separated in space. Having that separation opens totally new avenues to explore. He thinks that interest and advances in this wire technology are going to grow, but will require a lot of imagination for it to succeed. There needs to be more thinking done in terms of the separation of space and time, and the possibilities that presents for the next generation of fuels.

Tom Moore agreed. He pointed out that Nealson actually demonstrated in his talk that he had an aerobic side and an anaerobic side hooked together with a wire. "So you can do the chemistry that transcends 2 billion years of evolution, from a non-oxygenic to an oxygenic—you can combine them both with a wire. Better than that, the wire is a biological wire. It's just remarkable and fantastic, and wonderful to hear about," said Moore. He added that Nealson's work is a great illustration of successfully combining technology and natural systems to meet larger societal needs.

Microbial Fuel Cells

There was also a lot of discussion about uses of the nanowires, especially in microbial fuel cells, which have real potential for distributed application and to save money and decrease energy. Doug Ray said that these are great examples, potentially, of so-called appropriate technologies.

Moore talked about how microbial fuel cells could be deployed, even at this early stage, into the world, particularly Nealson's water purification system. Moore thinks it is not too soon to think about putting these technologies out there. They have one huge advantage over deploying higher technology systems, in that the question of translating a high-tech energy conversion system into a very low-tech, underdeveloped environment is challenging, for a number of reasons. For example, fuel cells and catalytic converters have a high risk of being stolen for their valuable materials. The advantage of the fuel cells that Nealson described in his talk is that they have no value except for what they do. The cells do not contain any valuable components such as metals, so they are less likely to be stolen. Yet, they can produce pure water.

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THE BIG PICTURE

Given the scale of energy needs, there was a lot of discussion about the ability of learning from biology to meet those needs. Many participants asked, in the long run and in the big picture, will biology really contribute to energy versus bioenergy? Doug Ray said that scientists often neglect to consider the importance of cost and scale of working with biological systems. For example, in terms of the global energy system, electricity from coal costs 4 cents per kilowatt-hour. It is very hard for other energy sources to compete with that, which is why coal is burned. Beating that price point is a challenge for everyone to consider.

Biology, Energy, and Sustainability

John Golbeck said that, in at least the next couple of decades, the big place for biology to contribute to energy is in understanding necessary land-use changes. Thauer added that in their breakout group the discussion was not that biology cannot contribute anything, but how much can it contribute? He said it may be small (1 or 2 percent), or it may be larger (10 percent), or larger still (50 percent). The other question is: Can it be done sustainably? If fertilizers are used, N_2O will be produced which is a 200-fold-times-more-efficient greenhouse gas than CO_2. That is not going to be sustainable.

Thauer said to consider his home country of Germany as an example. If Germany decided to take 100 percent of the area for agriculture and fertilize less—that might be more sustainable than devoting part of its land to make biofuels and continuing to fertilize. He said the numbers would be different for the United States. Germany has a population density of 239 per square kilometer, whereas United States has 30 people per square kilometer, and so the two countries will need to approach biomass development very differently. He said that, on average, per person, the United States has more biomass, and so, in principle, more options than the Europeans.

Judy Wall said her group also discussed the land-use issues highlighted by Rolf Thauer. For example, if all the available surface of the Earth could be used for biomass production, what is the maximum energy that it might be able to generate? Wall said that there are estimates that it is about 20 terawatts per year. However, based on the current population on Earth, the estimated need for energy 50 years from now is about 40 to 50 terawatts. That presents a large energy deficit to begin with—without accounting for population growth and other factors such as the land needed for houses, roads, and cities. Additional land is also needed for food, as well as to preserve wild habitats. The need for food reduces the amount of available land by about 50 to 70 percent more. Then, there is the consideration to leave some natural environment.

Wall also noted that a realistic contribution to energy from biomass is thus relatively small, on the order of perhaps 1 to 2 percent of the total budget. That raises the question then, is that large enough to worry about? She said yes, because the 1-2 percent translates into meeting the annual energy needs for somewhere between 3 million and 6 million people which is the size of a large city. Participants also discussed some of the current liquid biofuel production from cyanobacteria (Atsumi et al., 2009), which is making progress and looks as if it could make a significant contribution, on a per-hectare basis, relative to other biomass considerations as well. Wall said energy from biomass is not something to give up on, because every small contribution to that energy budget is going to be important.

Another issue discussed among workshop participants is with large-scale production of commodity chemicals using microbes. This presents a promising alternative to using fossil fuels for chemicals. However, right now it is not feasible to use it on a large scale. A good approach for now is to start with making specialty chemicals and then develop into the more bulk commodity chemicals.

Janet Westpheling commented that there has been at least one success in commodity chemicals. One of the real successes in metabolic engineering in *E. coli* is to make succinic acid for plastic production. She said there are manufacturing plants being constructed in the United States for that purpose. The process used is based on Lonnie Ingram's technology for making succinic acid in *E. coli*. This example illustrates that it is possible to economically use microbes to produce commodity-scale chemicals.

Maupin-Furlow agreed, but said that, for newly developed systems by startup companies, often it is better to start with a high-value specialty chemical and then go bulk. She said projects do not always fully think about how difficult it can be to produce chemicals on bulk scales.

It was mentioned that isolation of specific enzymes from microorganisms for specific applications is happening today. Several participants indicated that isolating and expressing enzymes is not much of a challenge anymore. The real interest and challenge is understanding more complex, perhaps emergent properties of enzymes and figuring out how to take advantage of those in the energy applications.

James Liao agreed that isolating enzymes from one organism and inserting into a foreign organism is a solved problem for the most part. He said that there is plenty of evidence of this in the current literature. However, he said that most people only demonstrate that the insertion produces a few micrograms or milligrams and call that a success. He said a microgram or milligram quantity is not going to burn in someone's engine. The real challenge is not to just show an enzyme can be made, but that it can be done in a high-flux way. There needs to be a goal of more than discovering enzymes and expressing them in different organisms. Consideration also needs to be given to throughput and scale.

Photosynthesis and Energy Storage

Photosynthesis was discussed as the main source of sustainable energy for the future. Judy Wall said that there are three main issues with photosynthesis: harvesting the energy, storing the energy, and converting the energy. For harvesting, photosynthesis works well for nature's purposes, but it is not as efficient as it could be for engineering devices for human needs. This is because Earth is not limited in light, for the most part, so it does not have to be particularly efficient. Thus, there is room to improve efficiency of the system.

Photosynthesis also presents a biological contribution to energy storage. The primary approach to energy storage is batteries. One problem is that current battery technology depends a lot on rare earth elements. James Liao added that the battery is also not a highly efficient storage material because the energy density is low. However, he said that it appears that chemical bonds are the most efficient way to store energy. For example, carbon-carbon bond or carbon-hydrogen bond would probably be the most practical energy storage in the near future. To store energy in liquid fuel, particularly biologically derived liquid fuel, the carbon-carbon bond formation ability provided by biological systems is probably the most unique aspect of biology's contribution to this energy problem.

One concern brought up by a participant is the energy density in biological products is also low, and will limit some of the applications. Also there are energy losses at every step away from the initial harvesting of light or energy. That has to be taken into consideration when looking to biology for inspiration for energy solutions.

The Human Element

Aside from the many scientific and technological issues discussed in the workshop, many participants said that, in the end, much of what happens in the future will ultimately depend on the actions of humanity. Many of the existing technologies available today could at least partially solve some energy problems. However, some participants noted that the implementation of those technologies is often driven by large political or economic forces. Education was discussed as one way to address this issue.

The impacts of lifestyle and energy use are also a huge factor in addressing the energy issue. Some participants asked: Should everything be left up to free markets, or does there need to be a set of stricter regulatory policies? There was acknowledgment among many participants that societal values need to be influenced to change the way energy is used, and to understand the importance of conserving energy. A participant commented that "we have to be careful what we implement, because once we are set on a course, it may or may not be reversible." For example, once U.S. farmers are paid subsidies to grow corn, will it be possible to go from corn ethanol to a different product?

Reference

Atsumi, S., W. Higashide, and J. C. Liao. 2009. Direct photosynthetic recycling of carbon dioxide to isobutyraldehyde. *Nat. Biotechnol.* 27(12):1177-1180.

A

Committee on Research Frontiers in Bioinspired Energy

Statement of Task

An ad hoc committee will explore the molecular-level frontiers of energy processes in nature through the development and implementation of an interactive, multidisciplinary, public workshop. They will prepare a report that will summarize the committee's assessment of what transpired at the event. The workshop will feature invited presentations and include discussion of key biological energy capture, storage, and transformation processes; gaps in knowledge and barriers to transitioning the current state of knowledge into applications; and underdeveloped research opportunities that might exist beyond disciplinary boundaries. The agenda for the workshop will be developed to focus on a variety of energy processes such as chemosynthesis, motility, and cellular functions, which serve as inspiring models for future energy systems. Emphasis in the presentations and discussion will be placed on molecular-level understanding rather than development of large-scale applications.

B

Workshop Agenda

Research Frontiers in Bioinspired Energy: Molecular-Level Learning from Natural Systems

January 6-7, 2011
The Georgetown University Hotel and Conference Center
3800 Reservoir Rd. NW
Washington, DC 20057

DAY 1

8:15 a.m.	Welcome and Overview
8:30 a.m.	**Opening Plenary Speaker:** Time scales and energy in biological systems **Leslie Dutton,** University of Pennsylvania
9:10 a.m.	Q&A

SESSION 1: ENERGY TRANSFORMATIONS

Covers: respiration, metabolism, chemical bonds, and molecules

9:30 a.m.	Origins of life and life in extreme environments **Penelope Boston**, New Mexico Tech
9:50 a.m.	Q&A
10:00 a.m.	Progress toward synthetic metabolisms **Steven Benner**, Foundation for Applied Molecular Evolution
10:20 a.m.	Q&A

10:30 a.m.	Break
10:45 a.m.	Breakout sessions
12:30 p.m.	Report back
1:30 p.m.	Lunch break

SESSION 2: ENERGY CAPTURE

Covers: antennae, membranes, ion channels

2:30 p.m.	Ion translocation across biological membranes **Janos Lanyi,** University of California, Irvine
2:50 p.m.	Q&A
3:00 p.m.	Novel mechanisms of anaerobic methane oxidation with sulfate **Rudolf Thauer,** Max Planck Institute
3:20 p.m.	Q&A
3:30 p.m.	Break
3:45 p.m.	Breakout sessions
5:30 p.m.	Report back
6:30 p.m.	Dinner break
7:15 p.m.	**Evening Plenary Speaker:** Solar energy harvesting in the epicuticle of the oriental hornet **Marian Plotkin,** National University of Singapore (remote)

DAY 2

SESSION 3: BIOINSPIRED ENERGY SYSTEMS

Covers: devices, engineered systems

8:00 a.m.	Energy production in biofuel cells **Kenneth Nealson**, J. Craig Venter Institute
8:20 a.m.	Q&A
8:30 a.m.	Microbes and the four basic strategies for life on Earth: What we can learn from what we know (and how to look for what we don't know) **Felisa Wolfe-Simon,** NASA and U.S. Geological Survey
8:50 a.m.	Q&A
9:00 a.m.	Breakout sessions
11:00 a.m.	Report back
12:00 p.m.	Lunch break
1:00 p.m.	**Closing Plenary Speaker:** Controlling nanoscale structure in 3D with an informational biopolymer: DNA **Nadrian Seeman,** New York University
1:40 p.m.	Q&A
2:00 p.m.	Closing remarks
2:30 p.m.	Adjourn

C

Biographical Information

COMMITTEE MEMBERS

Gregory A. Petsko (*Chair*) is the Gyula and Katica Tauber Professor of Biochemistry and Chemistry at Brandeis University, where he also directs the Rosenstiel Basic Medical Sciences Research Center. A Rhodes Scholar, Dr. Petsko was educated at Princeton, where he received his B.A. in chemistry in 1970, and at Oxford, where he received his Ph.D. in molecular biophysics in 1973. After a brief sojourn at the Institut de Biologie Physico-Chimique in Paris, he joined the faculty of Wayne State University, moving to the Massachusetts Institute of Technology in 1979. In 1990, he joined the Brandeis faculty. Dr. Petsko is a member of the National Academy of Sciences and the Institute of Medicine. He is working on time-resolved X-ray crystallography and related problems, with a particular emphasis on enzymology.

John Golbeck is a professor of biochemistry, biophysics, and chemistry at Pennsylvania State University (Penn State). He earned his B.S. in chemistry from Valparaiso University and his Ph.D. in biological chemistry from Indiana University. After working as an industrial scientist at Martin Marietta Laboratories, he resumed his academic career at the University of Nebraska and moved to Penn State in 1995. Dr. Golbeck's research interests focus on the assembly, structure, function, and modification of Type I photosynthetic reaction centers. His immediate research interests involve the protein and environmental factors that confer thermodynamic properties such as redox potentials to organic and inorganic cofactors, and the structural composition of Type I reaction centers from anaerobic photosynthetic bacteria, particularly heliobacteria. His long-term goal lies in engineering biohybrid photosynthetic constructs to produce hydrogen. Dr. Golbeck is a member of the American Biophysical Society and currently serves as the treasurer for the International Society of Photosynthesis Research.

James C. Liao is a Chancellor's Professor in the Department of Chemical and Biomolecular Engineering at University of California, Los Angeles (UCLA). Dr. Liao received his B.S. degree from National Taiwan University and his Ph.D. from the University of Wisconsin, Madison. After working as a research scientist at Eastman Kodak Company, Rochester, New York, he started his academic career at Texas A&M University in 1990 and moved to UCLA in 1997. He was elected a fellow of the American Institute for Medical and Biological Engineering (2002) and received numerous awards, including the National Science Foundation Young Investigator Award (1992), Merck Award for Metabolic Engineering (2006), Food, Pharmaceutical, and Bioengineering Division Award of the American Institute of Chemical Engineers (AIChE) (2006), Charles Thom Award of the Society for Industrial Microbiology (2007), Marvin Johnson Award of the American Chemical Society (2009), Alpha Chi Sigma Award of AIChE (2009), James E. Bailey Award of the Society for Biological Engineering (2009), and Presidential Green Chemistry Challenge Award (2010).

Julie Maupin-Furlow is a Research Foundation Professor in the Department of Microbiology and Cell Science at the University of Florida (UF). She is a microbial physiologist and biochemist with research expertise in archaea and the metabolic engineering of microbes for the production of renewable fuels and chemicals. Dr. Maupin-Furlow currently serves on the editorial boards of the *Journal of Bacteriology* and *Saline Systems*. She was co-chair of the 2009 Gordon Research Conference titled Archaea: Ecology, Metabolism & Molecular Biology, and is a current member of the UF Interdisciplinary Center for Biotechnology Proteomics Advisory Board, the Florida Center for Renewable Chemicals and Fuels, the Genetics Institute, the Center for Structural Biology, the Mass Spectrometry Users Group, and the Science for Life Howard Hughes Medical Institute Team. Maupin-Furlow has also served as a member of review panels for government agencies such as the National Science Foundation, the Department of Energy, the National Institutes of Health, and the National Aeronautics and Space Administration and as a panel manager for the U.S. Department of Agriculture National Research Initiative Biobased Products and Bioenergy Production Research Program 71.2. She received her B.S. degree in biology at Oral Roberts University and her Ph.D. in microbiology and cell science at the University of Florida.

Douglas Ray is the associate laboratory director for the Fundamental and Computational Sciences Directorate at the Pacific Northwest National Laboratory (PNNL), a role he has fulfilled since May 2006. The Directorate serves as the primary steward for research supported by the Department of Energy's Office of Science and the National Institutes of Health. As associate laboratory director, Dr. Ray directs more than 700 staff members in four research divisions: Atmospheric Sciences and Global Climate Change, Biological Sciences, Chemical and Materials Science, and Computational Sciences and Mathematics. Under his leadership, the divisions focus on important scientific problems with national implications as the key to advancing the frontiers of science. Dr. Ray has previously served as the interim deputy director for science and technology at PNNL where he was responsible for guiding the laboratory's overall capability development strategies, defining and advancing the laboratory's science and technology portfolio, coordinating its scientific discretionary investments, and integrating the laboratory's science and technology base to deliver essential scientific capability and accomplishments to advance the Department of Energy's missions. Dr. Ray earned a B.S. degree in physics from Kalamazoo College and a Ph.D. in chemistry from the University of California, Berkeley. He is a member of the American Chemical Society, American Physical Society, American Geophysical Union, American Association for the Advancement of Science, the Chemical Sciences Roundtable of the National Academy of Sciences, and the International Energy Agency's Experts Group on Science for Energy.

GUEST SPEAKERS

Leslie Dutton is currently a professor of biochemistry and biophysics at the University of Pennsylvania; chair, Department of Biochemistry and Biophysics (1994-2008); director, Johnson Foundation for Molecular Biophysics; and a Fellow of the Royal Society. Dr. Dutton completed his B.S. in chemistry (honors) at the University of Wales (1963), and his Ph.D. at the University of Wales (1967). The Dutton lab is interested in determining factors governing electron tunneling through natural proteins engaged in electron transfer, energy conversion, signaling, regulation, and enzyme redox catalysis. He is also involved in de novo design and synthesis of proteins engineered to perform natural functions such as electron transfer, proton translocation, charge-driven conformational changes, and redox catalysis in structured highly simplified settings.

Penelope Boston is director of the Cave and Karst Studies Program and associate professor in the Earth and Environmental Sciences Department at New Mexico Institute of Mining and Technology, in Socorro. Dr. Boston is also associate director of the National Cave and Karst Research Institute in Carlsbad, New Mexico. Her research areas include geomicrobiology and astrobiology in extreme environments (some of the world's most chemically and thermally extreme caves, hot and cold deserts, high latitudes and altitudes); human life support issues in space and planetary environments; and use of robotics to assist exploration and science in extreme Earth and extraterrestrial environments. Dr. Boston is author of over 130 technical and popular publications, editor of four volumes, and author of two upcoming popular books. Her work has been featured in over 100 print and broadcast media outlets over the past dozen years. She is a National Aeronautics and Space Administration (NASA) Institute for Advanced Concepts Fellow, and recently won the 2010 Lifetime Achievement in Science Award from the National Speleological Society. She is a past president of the Association of Mars Explorers (2006-2008). She holds M.S. and Ph.D. degrees from the University of Colorado, Boulder, an Advanced Study Program Fellowship at the National Center for Atmospheric Research, and a National Research Council postdoctoral fellowship at NASA Langley Research Center.

Steven A. Benner is a distinguished fellow at the Foundation for Applied Molecular Evolution (FFAME). Dr. Benner's research interests include chemical genetics, synthetic biology, paleogenetics, astrobiology, systems biology, and the connection of natural history to the physical sciences. His research group at FFAME initiated synthetic biology as a field and was the first to synthesize a gene for an enzyme and use organic synthesis to prepare the first artificial genetic systems. Dr. Benner's research has led to promising drug development leads through the invention of dynamic combinatorial chemistry, which combines ideas from different areas of chemistry and biology to discover small-molecule therapeutic leads. He also established paleomolecular biology, where researchers resurrect ancestral proteins from extinct organisms for study in the laboratory. Dr. Benner was a National Science Foundation graduate fellow, a Sloan Foundation fellow, recipient of the Nola Summer Award, Anniversary Prize of the Federation of European Biochemical Societies, and Sigma Xi Senior Faculty Award. He has also sat on numerous Space Studies Board committees, such as the Committee on the Astrophysical Context of Life, and the Committee on the Limits of Organic Life in Planetary Science. Dr. Benner received his B.S. and M.S. in molecular biophysics and biochemistry from Yale University, and his Ph.D. in chemistry from Harvard University.

Janos Lanyi attended Stanford (B.S. in 1959) and Harvard (Ph.D. in 1963), followed by postdoctoral training at Stanford (1963-1965, with Joshua Lederberg). Dr. Lanyi's professional experience includes research scientist at NASA-Ames Research Center (1965-1979), visiting scientist under the Alexander von Humbolt Foundation Program for Senior Scientists (1979-1980, with Dieter Oesterhelt), professor in the Department of Physiology and Biophysics, University of California, Irvine (1980-current), department chair (1995-2005), and the Peter Curran Memorial Lecture (Yale, 2001) and Ada Doisy Lectureship (University of Illinois, 2002). Lanyi's peer-reviewed publications, reviews, and book chapters number 300.

Rudolf K. Thauer is a biologist and a retired professor of microbiology who heads the Emeritus group at the Max Planck Institute for Terrestrial Microbiology in Marburg, Germany. Professor Thauer taught in the faculty of biology at the Philipps University in Marburg for about 15 years and is known primarily for his work on the biochemistry of methanogens. He received the Gottfried Wilhelm Leibniz Prize from the Deutsche Forschungsgemeinschaft in 1986, among numerous other honors including honorary doctorates from ETH Zurich, the University of Waterloo, and the University of Freiberg. In 1991, he became founding director of the Max Planck Institute for Terrestrial Microbiology in Marburg. A novel genus of betaproteobacteria was named *Thauera* in his honor.

Marian Plotkin is a research fellow at the University of Singapore, Department of Nanoscience and Nanotechnology Initiative, where his current research employs the optimization of cardiac cell therapy using injectable, resorbable, biocompatible materials. Dr. Plotkin received his B.S. in biomedicine from Tel Hai College (Israel), his M.S. in physiology from Tel Aviv University (Israel), and his doctorate in physiology from Tel Aviv University, where he led a joint project between Israeli and UK researchers that focused on the biophysical characterization of the yellow stripes in the oriental hornet cuticle, particularly concentrating on the special properties that allow the hornet to both absorb and then convert solar energy into activity. Dr. Plotkin's future research interests include the biomimicry of insect exoskeleton, oriental hornet silk and exoskeleton, and the biomimetic hydrogels for tissue engineering.

Kenneth Nealson received his B.S. degree in biochemistry (1965), and his Ph.D. in microbiology (1969), both from the University of Chicago. Dr. Nealson did postdoctoral work at Harvard University for 3 years. He then took a position at the Scripps Institution of Oceanography (University of California, San Diego), where he remained for 12 years, being promoted to professor of oceanography. During this time he studied aspects of marine bioluminescence, particularly the physiology and ecology of luminous bacteria and the organisms with which they are associated as symbionts. During this time, he elucidated the mechanism of signaling among bacteria, subsequently called quorum sensing. In 1980, utilizing a Guggenheim Fellowship for sabbatical leave, Dr. Nealson shifted his area of work to environmental microbiology and biogeochemistry, with a focus on the interactions between microbes and metals. In 1985, he took a position as the Shaw Distinguished Professor of Biology at the University of Wisconsin's Center for Great Lakes Studies, where he continued his studies of geobiology, with a focus on metals and microbes. This work has taken him to oceans, fjords, the Black Sea, the North American Great Lakes, and Lake Baikal, Russia. During the decade of the 1980s he was involved with the isolation and characterization of iron- and manganese-reducing microbes and elucidation of mechanism of extracellular electron transport. In 1997, Nealson moved to the Jet Propulsion Laboratory, where he directed the astrobiology group, set up the Center for Life Detection, and was program scientist for the Mars Sample Return (MSR) mission. In 2001, with the postponement of MSR, he moved to the University of Southern California, where he helped to establish the program in geobiology, and where he now resides as the Wrigley Professor of Environmental Sciences. Dr. Nealson's present work involves the study of biogeochemical processes in ultra-basic (i.e., $pH \geq 11.5$) environments, and extracellular electron transport as it relates to the cycling of iron and manganese oxides, as well as other insoluble components in sediments and other anoxic environments, and to the use of such bacteria both for bioremediation of toxic wastes, and for energy production in biofuel cells. Dr. Nealson is a member of the American Academy of Microbiology, serves on two National Research Council panels and three scientific advisory boards, and does extensive reviewing of proposals and programs.

Felisa Wolfe-Simon is currently a National Aeronautics and Space Administration (NASA) Astrobiology Research Fellow. Her interests broadly cover the intersection between biology and geology with a focus on astrobiology and the study of life in a planetary context. As an active member of the NASA Astrobiology Institute, Dr. Wolfe-Simon's research seeks to address geologically informed hypotheses to unravel the biogeochemical co-evolution of life and Earth. She applies tools from molecular biology, biochemistry, and physiology. Specifically, her work has addressed the evolution of metal-based enzymes and their role in globally relevant processes such as photosynthesis. Building on these ideas, she has developed an interest in using "what we do know" about biological processes to help uncover "what we don't know" and promote approaches to search for and think about alternative biochemistries on Earth. She obtained her dual undergraduate degrees in biology (B.A.) and music performance (B.M.) at Oberlin College and Conservatory of Music and went on to earn her Ph.D. in oceanography at Rutgers University. She pursued postdoctoral work as a National Science Foundation Fellow in Biology at Arizona State and Harvard Universities.

Nadrian C. Seeman was born in Chicago in 1945. After earning a B.S. in biochemistry from the University of Chicago, he received his Ph.D. in biological crystallography from the University of Pittsburgh in 1970. His postdoctoral training, at Columbia and the Massachusetts Institute of Technology, emphasized nucleic acid crystallography. He obtained his first independent position at the State University of New York at Albany, where his frustrations with the macromolecular crystallization experiment led him to the campus pub one day in the fall of 1980. There, he realized that the similarity between 6-arm DNA branched junctions and the flying fish in the periodic array of Escher's "Depth" might lead to a rational approach to the organization of matter on the nanometer scale, particularly crystallization. Since that day, he has been trying to implement this approach and its spinoffs such as nanorobotics and the organization of nanoelectronics. He has worked at New York University since 1988. When told in the mid-1980s that he was doing nanotechnology, his response was similar to that of M. Jourdain, the title character of Moliere's *Bourgeois Gentilehomme*, who was delighted to discover that he had been speaking prose all his life. He has published over 250 papers, and has won the Sidhu Award, the Feynman Prize, the Emerging Technologies Award, the Tulip Award in DNA Computing, the World Technology Network Award in Biotechnology, the NYACS Nichols Medal, the SCC Frontiers of Science Award, and the Kavli Prize in Nanoscience.

DISCUSSION LEADERS

Thomas Moore is a professor of chemistry and biochemistry at Arizona State University (ASU) and director of the Center for Bioenergy and Photosynthesis at ASU. Professor Moore worked under the direction of Professor Pill-Soon Song for the Ph.D. degree from Texas Tech University. He served as president of the American Society for Photobiology in 2004 and received the Senior Research Award from the society in 2001. Over the period of 2005-2007, Professor Moore was awarded a Chaire Internationale de Recherche Blaise Pascal, Région d'Ile de France. He teaches undergraduate- and graduate-level biochemistry at ASU and lectures on bioenergetics, energy, and sustainability at the CEA Saclay and Universitè de Paris Sud, Orsay. He has been awarded a visiting professorship at Vrije Universiteit, Amsterdam, for the summers of 2010 and 2011. He has served on several Department of Energy Basic Research Needs Workshops including the Basic Energy Sciences Grand Challenges Committee which produced *Directing Matter and Energy: Five Challenges for Science and the Imagination*, outlining research priorities for the foreseeable future. Professor Moore and his long-time colleagues, Professors Ana Moore and Devens Gust, collaborate on research in artificial photosynthesis, which is aimed at providing a deeper understanding of natural photosynthesis and the design, synthesis, and assembly of bioinspired constructs capable of sustainable energy production and conversion for human use.

R. David Britt is a chemistry professor at the University of California, Davis, whose research uses advanced electron paramagnetic resonance (EPR) techniques to probe structures and mechanisms of enzymes containing paramagnetic metal centers and/or radical intermediates. He received his Ph.D. in physics at the University of California, Berkeley (1988), working with Melvin P. Klein. A major focus of the Britt lab dates back to his graduate work, using pulsed EPR to study the manganese-containing oxygen evolving complex of Photosystem II. Other energy-relevant metalloenzymes under current study with EPR include ACS/CODH, nitrogenase, and hydrogenase. The Britt lab is also working to understand the chemistry of various water splitting synthetic catalysts, including the Kanan/Nocera self-assembling Co catalyst.

Judy Wall received her B.S. degree in chemistry from the University of North Carolina, Greensboro. Her Ph.D. is from Duke University in biochemistry with a specialization in genetics. Her dissertation explored the mechanism of generalized transduction by bacteriophage P1 in *Escherichia coli*. She then examined genetic exchange and nitrogen fixation in the phototroph *Rhodobacter capsulatus* at Indiana University during her postdoctoral training with Professor Howard Gest. In 1978, she joined the faculty of the Biochemistry Department at the University of Missouri, Columbia, where she resides today, and turned her attention to the genetics of the sulfate-reducing bacteria. She has served as a regular panel member for grant evaluations at the National Institutes of Health and the Department of Energy (DOE) and as an ad hoc reviewer on numerous panels. She is an American Association for the Advancement of Science fellow and a fellow of the American Academy for Microbiology where she has served two terms on the Board of Governors. She was editor and then editor-in-chief of the scholarly journal *Applied and Environmental Microbiology*. She has been generously funded through various programs of DOE for the past 23 years and was appointed to the DOE Biological and Environmental Research Advisory Board in 2009. She has mentored 17 graduate students, M.S. and Ph.D., 22 postdoctoral fellows, and numerous undergraduate students. In 2004, she received the Sigma Xi award for excellence in graduate research mentoring and, in 2008, received the Undergraduate Research Mentor Award. Her research area focuses on the genetics of the sulfate-reducing bacteria as a tool to explore energy generation in this anaerobe as it affects bioremediation capacities.

Robert Kelly received his B.S. and M.S. degrees in chemical engineering from the University of Virginia and, following 2 years at DuPont's Marshall Laboratory in Philadelphia, his Ph.D. in chemical engineering from North Carolina State University. He was formerly on the faculty in Chemical Engineering at Johns Hopkins University and is now in Chemical and Biomolecular Engineering at North Carolina State University, where he also directs the university's Biotechnology Program. His research program focuses on the biology and biotechnology of extremophilic microorganisms.

Janet Westpheling is an associate professor of genetics at the University of Georgia. She received her B.S. in microbiology from Purdue University (1973) and her Ph.D. in genetics from John Innes Centre, Norwich, England (1980). Dr. Westpheling's research interests include the rate-limiting step in the conversion of cellulosic material from crop plants such as poplar or switchgrass to simple sugars used for fermentation to ethanol in the recalcitrance of complex substrates, such as cellulose, xylan, and lignin, to simple mono- and polysaccharides. The focus of her research is to use functional and structural genomics-based methods, in conjunction with classical genetics and biochemical approaches, to identify novel biocatalytic (purified enzymes) and metabolic strategies (using whole cells) for bioenergy conversion. This research is part of a long-term collaboration between her lab and the laboratory of M. W. W. Adams in the Department of Biochemistry, focusing on the biotechnological potential of hyperthermophilic microorganisms and enzymes. Dr. Westpheling and her colleagues have developed genetic tools for manipulation of *Pyrococcus furiosus*, a hyperthermophilic fermentative anaerobic archaean capable of biomass conversion at or above temperatures of 100°C, and *Anaerocellum thermophilum,* a thermophilic, anaerobic Gram-positive bacterium, unique in its ability to efficiently utilize untreated cellulosic biomass. This work fits into the larger intellectual context of using classical (high-temperature microbial bioprocessing, large-scale protein purification) and modern (structural genomics, bioinformatics, transcriptional response analysis, gene replacement/mutational analysis) approaches to study extremophile biology and biotechnology as this relates to bioenergy conversion.

D

Workshop Attendees

First	Last	Affiliation	Role
Shibu	Abraham	Georgetown University	
Vernon	Anderson	National Institute of General Medical Sciences	
Mae Joanne	Aquila	Georgetown University	
Steve	Benner	FfAME	Speaker
Carol	Bessel	Department of Energy	
Penny	Boston	New Mexico Tech	Speaker
Robert	Boyd	Reporter	
R David	Britt	University of California, Davis	Discussion leader
Parag	Chitnis	Division of Division of Molecular Cell Biology, Directorate for Biological Sciences, National Science Foundation	
Steven	Chuang	University of Akron	
Richard	Conroy	National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health	
Bruce	Diner	E. I. du Pont de Nemours & Co.	
Carmen	Drahl	*Chemical & Engineering News*	

First	Last	Affiliation	Role
Leslie	Dutton	University of Pennsylvania	Speaker
Lars	Friberg	Swedish Embassy	
Raymond	Gephart	Georgetown University	
John	Golbeck	Pennsylvania State University	Committee member
Elias	Greenbaum	Oak Ridge National Laboratory	
Yanfeng	Gu	Chinese Embassy	
Kerry	Hamilton	Environmental Protection Agency	
Terri	Hanks	Booz Allen Hamilton	
Chad	Haynes	Booz Allen Hamilton	
William	Hickey	Self	
Terance	Hilsabeck	General Atomics	
Pete	Kelly	No current affiliation	
Robert	Kelly	North Carolina State University	Discussion leader
Janos	Lanyi	University of California, Irvine	Speaker
Nikolai	Lebedev	Naval Research Laboratory	
Audrey	Levine	EPA	
James	Liao	University of California, Los Angeles	Committee member
Delina	Lyon	Self	
Julie	Maupin-Furlow	University of Florida	Committee member
Raul	Miranda	Department of Energy Basic Energy Sciences Office	
Thomas	Moore	Arizona State University	Discussion leader
Ken	Nealson	J. Craig Venter Institute	Speaker
World	Nieh	U.S. Forest Service	
Joe	Palca	National Public Radio	
Parag	Parekh	National Science Foundation	
Bradley	Perrin	Georgetown University	
Gregory	Petsko	Brandeis University	Committee member
Aga	Pinowska	General Atomics	
David	Pittman	Freelance journalist	
Doug	Ray	Pacific Northwest National Laboratory	

First	**Last**	**Affiliation**	**Role**
Anne	Riederer	American Association for the Advancement of Science Fellow with the Environmental Protection Agency	
David	Rockcliffe	National Science Foundation	
Greg	Rorrer	National Science Foundation	
Bijay	Sarkar	Georgetown University	
Nadrian	Seeman	New York University	Speaker
Daniel	Seidenberg	Georgetown University	
Suruchi	Shrestha	Salem University	
Robert	Stack	Office of Science, Department of Energy	
Veronika	Szalai	National Institute of Standards and Technology	
Rudolph	Thauer	Max Planck Institute	Speaker
David	Thomassen	Biological and Environmental Research, Department of Energy	
YuYe	Tong	Georgetown University	
Emily	Viau	Stanford University	
Val	Vullev	University of California, Riverside	
Judy	Wall	University of Missouri	Discussion leader
Alexander	Weppelman	Environmental Protection Agency	
Mitzi	Wertheim	Cebrowski Institute	
Janet	Westpheling	University of Georgia	Discussion leader
Felisa	Wolfe-Simon	National Aeronautics and Space Administration Astrobiology Fellow	Speaker