The Future of Energy from a Biological Engineer's Perspective by Justin Buck and Marcio von Muhlen¹

jrbuck@mit.edu e marciovonm@mit.edu
July, 2006

The challenge of supplying the world with cheap, abundant and reliable energy will no doubt mark the history of the 21st century. Momentarily sidelined by 10 dollar barrels of crude oil in the 1990's, the recent run-up in oil prices coupled with the increasingly appreciated oncoming crisis of global warming demands serious consideration for alternative and still highly speculative energy solutions. We believe that maturing technologies in genetics and molecular biology are opening the door for highly efficient biologically inspired energy sources. Humans have in a way exploited biological fuels for all of recorded history, burning organic materials from renewable (biomass) to limited (fossil fuels) whose primary origin could be traced to solar energy. At present we are in large part dependent on these biological mechanisms that transform solar energy to more useful forms, supplanted in part by modern energy converters such as solar panels, hydroelectric plants and a few solar-independent sources such as nuclear fission.

Regrettably, these alternative sources for various economic and environmental reasons have failed to compete with fossil fuels as primary energy sources for human activity. Solar energy is widely accepted as being the most abundant and practical renewable energy source available with a yearly average of 1780 000 terawatts (TW) incident on the planet, compared to global human consumption of 13 TW¹. The high costs associated with mining and refining the materials necessary for solar panels (mainly high purity silicon) are what has prevented their widespread acceptance – current direct solar energy technologies provide energy at a cost 4 to 5 fold higher than present sources. As the costs associated with fossil fuels increase, both directly in production

expenses and indirectly in the effects of global warming and air pollution, it's clear that eventually we will be forced to rely on alternative energy sources. We believe that scientists can accelerate this transition by lowering the costs of alternative energies. It's unreasonable to expect that the indirect costs of fossil fuels will be taken into account by industrial or political forces; the failure of the Kyoto Protocol to be accepted by the largest current energy user (the USA) and the likely largest future energy user (China) indicates that short-term global economic forces have thus far proven more powerful than any potentially far-sighted political organization (the United Nations, climate conferences) can be.

So what can biological engineering bring to the table? Incremental improvements to crop yields through genetic engineering have been a reality for many decades, consisting mainly of insertion of pesticide resistant genes (see Gurr 2005 for a recent review²). It should be noted that genetically modified organisms (GMOs) have faced varying degrees of acceptance in traditional agriculture for human consumption; concerns over the effects of GMO crops on nutrition and the surrounding ecosystem are ongoing (perhaps most famously in the debate over Chapela and co-workers fiercely disputed reports of gene-hopping in Mexican maize^{3,4}). We will avoid this debate here, instead focusing on the positive possibilities of increasingly refined techniques. The recent pace of biological innovation has the potential for a radical re-thinking of the extent to which biological photosynthetic machinery can be manipulated. Modifications at the amino-acid level of specific photosynthetic proteins as well as insertion of hydrogen and hydrocarbon generating enzymes allow for creation of custom-tailored

¹The authors are currently PhD track students in the Division of Biological Engineering at the Massachusetts Institute of Technology

organisms, most likely microbial or algae, with potential for massive improvements in energy conversion efficiencies, hopefully to such a level as to be able to compete economically with fossil fuels. We will briefly discuss the current energy landscape, then introduce various aspects of photosynthetic organisms where current and future research can lead to an improved biological energy source. Although we see a lot of potential in this research, its worth to caution that practical results should not be expected for a decades long timeline.

Current Energy Use: Quick Facts and a Looming Crisis

In order to boil down such a massive field to a few paragraphs we are necessarily brief. We borrow largely from a much larger and informative review¹ also focused on bioenergy. Global demand for energy is predicted to rise from 13 TW in 2000 to ~46 terawatts in 2100. As can be seen in table 1, at such rates the current proven global energy supplies would be extinguished in ~40-50 years. It should be noted that these are proven reserves at today's market prices; increasing price drives further exploration and mining of more costly supplies not counted in these numbers.

In addition to declining supply, global warming presents another significant barrier to continued reliance on fossil fuels as our preferred energy source. Atmospheric CO₂ levels are the principal indicator of human induced pro-warming effect. Stable for 400 000 years between 200 and 280 ppm, recently the level has risen sharply to 370 ppm. Climate predictions at the 550 ppm level include mass extinctions and 4-6 meter sea level rises^{5,6}; maintaining levels below 450 ppm is forecast to require half of energy use in 2025 to be carbon neutral⁷. This would require carbon neutral energy sources at roughly the same capacity as today's entire energy industry (~10 TW) to be erected in less than 20 years, a daunting and likely unachievable goal.

A system as complex as global climate is expected to be difficult to understand, much less predict on a decades long time course.

However it is telling that even a major petroleum companies such as British Petroleum agrees that the problem is real and growing. In a 1997 speech, John Browne, the CEO of BP declared "...there is now an effective consensus among the world's leading scientists and serious and well informed people outside the scientific community that there is a discernible human influence on the climate, and a link between the concentration of carbon dioxide and the increase in temperature"⁸. A visit to the website of BP today (www.bp.com) reveals at the very least an effort to promote alternative energy and address atmospheric carbon build-up. Information on this subject is wide-ranging and often disputed, but principally agreed-on conclusions have emerged again and again in top-level articles in Science and Nature as those referenced here. Possible solutions to the problem range from conservation (often quoted, but difficult to enforce on a mass scale) to more exotic possibilities such as forming clouds to reflect more sunlight to space, or parking a massive mirror in orbit for the same purpose⁹. We expect the most likely and in many ways partial solution will be greater expenditures on alternative, carbon-neutral energy sources. We now focus our attention in detail on how nature has evolved its primary energy solution over billions of years, which may provide a blueprint for our own energy needs.

Photosynthesis

Photosynthesis is the phenomena by which autotrophic organisms capture the energy of sunlight to power their metabolism and fix their atomic building blocks carbon and nitrogen from atmospheric sources. It can be readily appreciated that photosynthetic processes are ultimately responsible for the sustenance of (almost) all life in our planet, and for the existence of oxygen in our atmosphere. The wide variety of organisms which perform photosynthesis rely on distinct biochemical processes depending on phyla and ecological niche, with the common feature of using photons from sunlight to excite electrons in a photosystem (PS) reaction center which are in turn shuttled by mobile carriers in an electron transport chain. The reduced reaction center then become a strong oxidant capable of stripping electrons from environmentally

available substrates. Extracted protons are concurrently shuttled across the thylakoid membrane, the lipid bilayer that anchors and stabilizes the PS, to generate a proton gradient suitable for the activity of ATP synthase. We will focus on oxygenic organisms employing two distinct photosystems, termed PSI and PSII, and water as a substrate, which includes all eukaryotic autotrophs and the prokaryotic cyanobacteria. We will briefly detail how this system functions in preparation for our discussion on co-opting specific subsystems for more direct human purposes.

The Z-Scheme

Figure 1 demonstrates a clear advantage of using dual photosystems; the system acts as a two-stage energizer that ultimately enables the reduction of NADP^+ to NADPH, which subsequently provides the reducing power for the carbon fixing dark reactions. Furthermore the generation of ATP and NADPH are separated and their relative production rates can be modulated by the organism as dictated by metabolic needs. Of particular importance to our purposes is the compartmentalization of the sub-processes depicted in Figure 1. Photon absorption occurs in the chlorophyll pigment molecules located in the reaction center, and also in accessory light harvesting complexes (LHC) that surround the photosystems and serve to expand the surface area for harvesting photons. The transfer of high-energy electrons from PSII to PSI proceeds via inter-membrane quinones to the cytochrome b_6 -f complex, then via cytosolic plastocyanins to PS1. In prokaryotes the inner plasma membrane functions as the thylakoid membrane, and is especially fluid with a higher than normal content of unsaturated fatty acid chains.

Photosystem II

We now focus on PSII, site of the oxygen evolving complex (OEC) and relevant reactions (reactions 1-6). The PSII complex is a membrane bound and highly complex dimer, containing at least 20 protein subunits, 77 cofactors and 7 integrally bound lipids per ~750 kDa monomer (Figure 2). Complex assembly *in vivo* has been found to utilize distinct mechanisms termed “assisted” and

“spontaneous”¹⁰ in regards to dependence on signal recognition particles in subunit domains. Reaction 1 occurs in the OEC, while reactions 2-6 occur in the PSII reaction center (Figure 2). The plastoquinone Q_B is twice reduced (not shown), abstracts 2 protons from the cytosolic side of the thylakoid membrane (opposite the OEC) then diffuses inside the membrane to cytochrome b_6 f. Reactions 2-6 are essentially irreversible due to large redox potential drops between reactants and products. In summary, PS2 takes as inputs solar radiation and H_2O and outputs molecular oxygen and high reduction potential quinones which spatially localize protons. Proton gradients are used to turn ATP synthase and high reduction potential quinones reduce NADP^+ . Protocols for genetic engineering and subsequent analysis of complex formation, efficiency and cofactor redox potentials are widespread in recent scientific literature^{11,12} in particular for the cyanobacteria *Thermosynechococcus elongatus* (whose photosynthetic apparatus is similar to that of multicellular plants). The complete genome of *T. elongatus* became available in 2002 in the easily accessible Kyoto Encyclopedia of Genes and Genomes (KEGG) database¹³. As *Escherichia coli* and *Saccharomyces cerevisiae* became the workhorse for microbial production of therapeutic proteins, we believe *T. elongatus* and similar cyanobacteria may become the workhorses for microbial energy related research since they unicellular but contain generally the same photosynthetic machinery as higher plants.

Crucial to an understanding of how the reactions and processes detailed above actually occur in the photosynthetic system is their spatial localization. Attempts at X-ray crystallography of PSI and PSII have been slow in coming due to the difficulty in crystallizing the lipid-bound, multi-subunit complexes. However recent gains show promise in understanding the photosystem complexes at the molecular level. Loll and co-workers published a 3 angstrom structure in 2005 with complete cofactor arrangement¹⁴. Many labs throughout the world are actively deciphering the chemistry of photosynthesis. Once the functions and characteristics of the many parts are well-enough understood, biological engineers may

begin to appropriate them for generating organisms capable of efficiently transforming solar energy to a form more easily used by human beings. A key realization is that nature's photosystems are exquisitely adept at performing functions beneficial to the organism because evolutionary forces drove them to do so. Modern genetic techniques allow the redesign of such evolutionary forces on the bench-top so that they result in functions more directly beneficial to us. We will discuss some relevant classes of molecules in this effort, and then briefly mention three research groups undertaking exactly this sort of research.

Hydrogenases

With the prospect of increased fuel cell efficiency and possible transition to a hydrogen-based economy, biological metabolism of hydrogen is an interesting topic. For hydrogen to become a low-cost fuel source, there must be a way to split water and evolve hydrogen and oxygen at low temperatures and pressures and without the use of electrical current. In the process of photosynthesis, photosystem II provides a means for extracting electrons from water and releasing molecular oxygen and hydrogen ions. If the hydrogen ions and electrons derived from water can be efficiently recombined to produce molecular hydrogen it would be a tremendous advancement for the energy industry¹⁵.

Luckily, nature has already produced a solution for this problem. A hydrogenase is an enzyme that catalyzes the reduction of two protons to molecular hydrogen or the reverse oxidation of molecular hydrogen to release two protons into solution and two electrons to an oxidizing agent. The general reaction of a hydrogenase can be written as $2\text{H}^+ + 2\text{e}^- \leftrightarrow \text{H}_2$

Hydrogenases are found naturally in many different organisms, and an organism may have multiple hydrogenase genes serving various different roles. They can be localized to a membrane or floating free in solution. Hydrogenases can be classified into two groups based on their functionality; uptake hydrogenases consume molecular hydrogen and provide reducing agents for cellular

processes; reversible hydrogenases catalyze the reaction in either direction to store or liberate energy depending on the needs of the cell¹⁶.

Hydrogenases are notorious for being extremely sensitive to oxygen. This is correlated to the primary use of hydrogenases under anaerobic conditions. This is a significant obstacle to combining hydrogenase activity with water splitting and subsequent oxygen evolution. Engineering hydrogenases with increased tolerance to oxygen is an active field of research. A typical approach to creating an oxygen tolerant hydrogenase is to limit the accessibility of molecular oxygen to the metal active site (usually either Fe-Fe or Ni-Fe) of the enzyme. This is typically achieved with a pore size that excludes oxygen while permitting the passage of molecular hydrogen¹⁶.

Rhodopsins

In addition to photosystems, nature has another paradigm for the collection of electromagnetic radiation, rhodopsins. Rhodopsins are transmembrane proteins which bind a chromophore or pigment (often retinal) in much the same fashion as photosystems bind chlorophyll. The pigment is responsible for the absorption of light of the rhodopsin. When the photon is absorbed, a photoisomerization occurs within the pigment causing a conformation change in both the pigment and the rhodopsin. This conformational change allows the transport of ions across the membrane. In the case of some forms of rhodopsins found in bacteria, bacteriorhodopsins and proteorhodopsins, the result of the photoisomerization is the transport of a hydrogen ion across the membrane. The electrochemical gradient formed by the unidirectional transport of hydrogen ions, like that formed by the light reactions of photosynthesis, can be used by an ATP synthase protein to produce usable energy. In contrast to photosystem operation, this process does not split water, releasing molecular oxygen and hydrogen ions, or produce any high reduction potential molecules which can be used for a carbon fixation process. This process does, however, offer another model for the efficient capture of solar radiation and the subsequent storage in a high energy chemical bond which may play a significant role in global

phototrophy^{17,18}.

Bioethanol/Biodiesel

Biology has developed several pathways to store and later utilize energy the energy harnessed from photosynthesis, most of which involve the metabolism of simple sugars. The same high energy molecules utilized in biological systems can be and are used for energy on an industrial scale. In addition to burning biomass to produce steam and electricity, there are two major liquid fuels that can be derived from biomass material, ethanol and diesel¹⁹. We will briefly describe the bioethanol and biodiesel processes and a few ways in which there is research to improve this technology.

Bioethanol is produced from the fermentation of monosaccharides by yeast and bacteria. In the absence of oxygen, alcohol fermentation is the only sustainable pathway for energy production for these organisms, which are able to survive on the limited amount of energy liberated during the conversion of one molecule of glucose to two molecules of ethanol and two molecules of carbon dioxide ($C_6H_{12}O_6 \rightarrow 2 C_2H_5OH + 2 CO_2$). Industrial scale bioethanol production is achieved by delivering a high sugar feedstock to an anaerobic bioreactor composed of suitable yeast and/or bacteria. The ethanol extracted from the reactor is purified by conventional chemical refining technologies to produce the fuel grade ethanol. The feedstock delivered to reactors is derived from a biomass source, typically sugar cane or corn. However, plants do not keep a large quantity of monosaccharides available at all times; most of the carbohydrates in the plants are stored in starches for long term energy reserves or in cellulose for structural purposes. Therefore, the carbohydrates extracted from the harvested biomass must be processed prior to use as fermentation feed stock. This is done through a combination of acid and enzyme catalysis which breaks the bonds of the polysaccharide molecules and releases the monosaccharides^{19,20}.

Biodiesel is produced from the lipid components (fats and oils) of the produced biomass. In the native organism, these

compounds serve as energy reserves as well as providing critical structural functions, most notably compartmentalization within the cell. The long aliphatic hydrocarbon chains composing these fats and oils are the equivalent of the long hydrocarbons found in petroleum. Small hydrocarbon chains (lights) in petroleum are used to make gasoline and other organic chemicals, while the long chains (heavies) are used for diesel fuel. Since long chains are abundant in biologically produced fats and oils, diesel is an ideal choice for a liquid fuel product. As with bioethanol, the production of biodiesel requires processing and purification of the biomass to produce a useable fuel product^{19,20}.

There are many active areas of research to improve the yield and efficiency of these energy harvesting technologies, here we only mention three areas of research in improving biomass technologies. One approach is to genetically modify and design the source crops to grow faster and with larger mass yields. Switchgrass is a promising alternative to corn for the production of ethanol because of its rapid growth and relatively minimal soil nutrient depletion. Another means of improving these technologies is to improve the efficiency of the biological processing methods, thereby reducing the amount materials and energy required to degrade complex carbohydrates into simple sugars or extract oils from plants. A third area of research which will make these biomass technologies more cost effective is the continued improvement of traditional chemical purification processes, particularly energy intensive and cost inhibitive distillation²¹.

Biohydrogen

As we've mentioned before, a variety of groups are pursuing research towards biologically inspired energy sources. We highlight three here, but there are many others. A group called biohydrogen generation at Stanford University is focusing on improving oxygen tolerance for hydrogenases, currently a critical shortcoming to practical hydrogen generation²². Princeton along with four other major universities has an effort called biosolar whose first goal is characterization of different hydrogenase enzymes from a variety of

microbial sources²³. Finally, Ihara and co-workers recently reported a functional PSI/hydrogenase fusion protein, with the electron transport chain of PSI from *Thermosyneschococcus. elongatus* directly linked to the active site of a hydrogenase from *Ralstonia eutropha* via a glycosidic linkage²⁴. Hydrogen yields were extremely low, but the ability to generate a working hybrid protein with detectable energy output signals a milestone in the application of genetic techniques to manufacture useful machines at the molecular level (see figure 3).

Conclusion

The era of cheap fossil fuel based energy is seemingly close to an end, and practical alternatives should be studied with a reasonable sense of urgency. The tools of modern biology have enabled the engineering of microorganisms to affordably perform complicated chemical syntheses at the backbone of the biotechnology industry. We believe the same approach can be applied to the energy industry. By utilizing the energy collecting and processing machinery present in living cells in a novel way, biology may provide a solution to our looming energy crisis.

References

1. Kruse O, Rupprecht J, Mussnug JH, Dismukes GC, Hankamer B. Photosynthesis: a blueprint for solar energy capture and biohydrogen production technologies. *Photochem. Photobiol. Sci.* 4, 957 – 970 (2005)
2. Gurr SJ, Rushton PJ. Engineering plants with increased disease resistance: what are we going to express? *Trends Biotechnolog.* 23(6):275-82. (2005)
3. Quist D, Chapela IH. Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. *Nature* 414(6863):541-3. (2001)
4. Marris, E. Four years on, no transgenes found in Mexican maize. *Nature* 436(7052):760. (2005)
5. O'Neill BC, Oppenheimer M. *Science* 296, 1971–1972. (2002)
6. Hughes TP, Baird AH, Bellwood DR, Card M, Connolly SR, Folke C, Grosberg R, Hoegh-Guldberg O, Jackson JBC, Kleypas J, Lough JM, Marshall P, Nystrom M, Palumbi SR, Pandolfi JM, Rosen B, Roughgarden J. *Science* 301, 929–933. (2003)
7. Hoffert MI, Caldeira K, Jain AK, Haites EF, Harvey LDD, Potter SD, Schlesinger ME, Schneider SH, Watts RG, Wigley TML, Wuebbles DJ. Energy implications of CO₂ stabilization. *Nature* 395, 881–884. (1998)
8. Browne, J. Climate Change. Stanford University, 19 May 1997. http://www.gsb.stanford.edu/community/bmag/sbsm0997/feature_ranks.html, accessed on July 16, 2006.
9. Broad, W. How to Cool a Planet (Maybe). *The New York Times*, June 27, 2006.
10. Baena-Gonzalez E, Aro EM. Biogenesis, assembly and turnover of photosystem II units. *Phil. Trans. R. Soc. Lond. B* 357: 1451–1460 (2002)
11. Kern J, Loll B, Luneberg C, DiFiore D, Biesiadka J, Irrgang KD, Zouni A. Purification, characterisation and crystallisation of photosystem II from *Thermosynechococcus elongatus* cultivated in a new type of photobioreactor. *Biochim Biophys Acta*, 1706(1-2):147-57 (2005).
12. Gibasiewicz K, Dobek A, Breton J, Leibl W. Modulation of Primary Radical Pair Kinetics and Energetics in Photosystem II by the Redox State of the Quinone Electron Acceptor QA. *Biophysical Journal*, 80:1617-1630 (2001).
13. Kanehisa, M. and Goto, S.; KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Res.* 28, 27-30. (2000)
14. Loll B, Kern J, Saenger W, Zouni A, Biesiadka J. Towards complete cofactor arrangement in the 3.0 Å resolution structure of photosystem II. *Nature*, 15;438(7070):1040-4 (2005).
15. Mertens R, Liese A. Biotechnological applications of hydrogenases. *Current Opinion in Biotechnology.* 15:343-348. (2004)
16. Tamagnini P, Axelsson R, Lindberg P, Oxfelt F, Wunschiers R, Lindblad P. Hydrogenases and Hydrogen Metabolism of Cyanobacteria. *Microbiology and Molecular Biology Reviews.* 66(1):1-20. (2002)
17. Béjà O, Aravind L, Koonin EV, Suzuki MT, Hadd A, Nguyen LP, Jovanovich SB, Gates CM, Feldman RA, Spudich JL, Spudich EN, DeLong EF. Bacterial Rhodopsin: Evidence for a New Type of Phototrophy in the Sea. *Science* 289:1902-6. (2000)
18. Béjà O, Spudich EN, Spudich JL, Leclerc M,

- DeLong EF. Proteorhodopsin phototrophy in the ocean. *Nature* 411:786-9. (2001)
19. http://gcep.stanford.edu/research/factsheets/biohydrogen_generation.html
 20. <http://www1.eere.energy.gov/biomass/>
 21. Rostrup-Nielsen JR. Making Fuels from Biomass. *Science* 308(5727):1421-1422
 22. Ragauskas AJ, Williams CK, Davison BH, Britovsek G, Cairney J, Eckert CA, Frederick WJ Jr., Hallett JP, Leak DL, Liotta CL, Mielenz JR, Murphy R, Templer R, Tschaplinski T.
 23. The Path Forward for Biofuels and Biomaterials. *Science* 311(5760):484-489. (2006)
 24. <http://www.princeton.edu/~biosolar/>
 25. Ihara M, Nishihara H, Yoon K, Lenz O, Friedrich B, Nakamoto H, Kojima K, Honma D, Kamachi T, Okura I. Light-Driven Hydrogen Production by a Hybrid Complex of a [NiFe]-Hydrogenase and the Cyanobacterial Photosystem I. *Photochemistry and Photobiology*, [Epub ahead of print] Jan. 1 (2006).
 26. *World Energy Outlook 2001. Insights Assessing Today's Supplies to Fuel Tomorrow's Growth*, International Energy Agency, Paris, 2001.
 27. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. *Molecular Biology of the Cell* (4th edition). Garland Science, New York. 2002.
 28. Guex N, Diemand A, Peitsch MC. Protein Modelling for All. *Trends Biochem. Sci.* 24 (9): 364-367 (1999).
 29. Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH. *MMDB: Entrez's 3D-structure database. Nucleic Acids Res.* 31(1): 474-7. (2003)

Reactions

Figures

Fuel	Proven Reserve	Energy Equivalent
Crude Petroleum	1-1.25 Trillion Barrels	540 TW*year
Natural Gas	6-7 Quadrillion SCF	580 TW*year
Coal	1 Trillion Short Tons	1000 TW*year

Table 1. Proven Reserve as reported by the Energy Information Administration (EIA, <http://www.eia.doe.gov/>) and Energy equivalent as reported by the International Energy Agency²⁵. (SCF is Standard Cubic Feet, TW = terawatt).

Figure 1: The Z-scheme for dual photosystem organisms (for a thorough description refer to Molecular Biology of the Cell by Alberts et al²⁶ or any comprehensive Biochemistry textbook).

Figure 2 – Entire photosystem II (PSII) dimer complex. Monomer in the left in white, monomer in the right with coloring by subunit. Transmembrane view (cytosol above, luminal space below) showing transmembrane helices in the top half, oxygen evolving center in the bottom half of the complex. Image generated with DeepView²⁷. The PSII structure shown is 2AXT from Loll, 2005¹⁴, accessible in the Research Collaboratory for Structural Bioinformatics Protein Databank²⁷.