

Liquid Sunlight: The Evolution of Photosynthetic Biohybrids

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Liquid sunlight can be considered as a new form of chemical energy converted and stored in chemical bonds from solar energy.¹ Natural photosynthesis in green plants represents one of the most elegant and powerful examples of such a process. As the only energy input into the ecosphere, solar energy positions itself as one of the most promising solutions to address the crisis on the environment and climate change. Efficient capture and storage of solar energy can provide unlimited renewable power sources and drive the capture and conversion of greenhouse gases such as CO₂ into valuable chemicals. Such an artificial photosynthetic process presents one of the most important solutions, if not the only one, toward net-zero carbon emission or even negative emission society in the near future.

Despite intense chemistry, biology, materials, and engineering efforts to develop such CO₂ fixation processes, comprehensive solutions remain elusive. Traditionally this CO₂ fixation problem has been approached with either a purely synthetic material approach or biological approach. Biology achieves CO₂ fixation to energy-dense multicarbon compounds with impressive selectivity yet suffers from relatively poor photosynthetic light absorption. The synthetic material method typically uses light-absorbing semiconductors coupled with synthetic catalysts for solar-driven CO₂ reduction; however, the progress of this approach is hampered by the lack of a suitable synthetic catalyst with sufficient activity and selectivity, especially for multicarbon compounds.

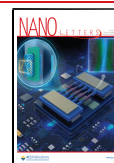
In this regard, our recent work focuses on a novel cyborgian design for solar fuel production: the introduction of photosynthetic biohybrid systems (PBSs)² for photochemical biosynthesis using CO₂ where the optoelectronic properties of inorganic material systems are integrated with the self-regenerative properties of biological systems.^{3–5} Biological microorganisms engage a collection of enzymes and reductive pathways to produce long-chain hydrocarbons from simple building blocks, including CO₂, N₂, and H₂O. Enzymes and proteins within the metabolic pathways of the living cells benefit from an embedded building code in genetic information and can be repaired and replicated as necessary. For these reasons, the integration of inorganic light-harvesters and whole-cell biocatalysts would take advantage of the best functions of each component. In particular, semiconducting nanomaterials are highly configurable with tunable broadband light absorption and surface charge, pair well with microorganisms and enable significant sunlight capture, hence solar-to-chemical conversion.

It has been demonstrated that by using an autotrophic homoacetogen *Sporomusa ovata* or *M. thermoacetica* as a CO₂-reducing electrocatalyst, it is possible to couple these microorganisms with silicon nanowires, CdS quantum dots, and gold nanoclusters for solar-powered CO₂ reduction.^{6–8} Under solar irradiation, the integrated biohybrids could effectively capture sunlight and reduce CO₂ into a common chemical intermediate, acetic acid, without additional external energy input, and the energy conversion efficiency could be as high as 3.6%.⁹ This solar-to-chemical energy conversion efficiency can be further optimized by tuning the medium pH, semiconductor bandgap, microorganism selection, the semiconductor and bacteria loading density, as well as the charge transfer interface. In addition, several other microorganisms including *R. eutropha*, *S. cerevisiae*, *A. vinelandii*, and *C. necator* have also been introduced into this biohybrid family.^{10–12}

It is well-established that many microorganisms survive and thrive as a diverse community.¹³ They can readily exchange metabolic byproducts among different species in a shared living environment. The formation of a synergistic consortium with multiple microorganisms could minimize the loss in metabolites exchange, representing a highly effective energy exchange pathway in nature. It also offers another vital strategy to upgrade the chemical intermediates photochemically produced from CO₂. For example, solar-generated acetate from *S. Ovata* biohybrids can be directly fed to *E. coli* or other heterotrophic microorganisms, which can convert acetate into value-added multicarbon products, such as *n*-butanol, polyhydroxybutyrate polymer, and other natural products.⁶

Typically photosynthetic biohybrids development would encompass several essential aspects: selection of a suitable microorganism for chemical synthesis; selection of a semiconductor nanomaterial as photosensitizer; examination of the synergistic effects of the inorganic–biological hybrid system; and study of the fundamental mechanisms at the newly formed biotic–abiotic interfaces for energy transduction and molecular

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formation. The evolution of such photosynthetic biohybrids is a direct result of the photosensitization of a nonphotosynthetic microorganism. The microorganism can carry out regular or enhanced metabolic function through this new form of energy transduction enabled by inorganic photosensitizers. These photosynthetic biohybrids boost the best functions of biological whole-cell catalysts and semiconducting nanomaterials. However, the fundamental electron transfer and energy transduction pathway in these emerging photosynthetic biohybrids remains largely unexplored due to the complex nature of the biotic/abiotic semiconductor/bacteria interfaces.

Liquid sunlight represents a new form of chemical energy converted and stored in chemical bonds from solar energy. Photosynthetic biohybrids produce liquid sunlight through a “photon-in, chemical bond-out” materials/biology interface that can be probed through spatiotemporal imaging, spectroscopic, transcriptional, and proteomic analyses.

Fundamentally, the photosynthetic function of these PBSs originates from a “photon-in, chemical bond-out” materials/biology interface that spans multiple orders of magnitude both in the length and time scale (Figure 1). To probe such a “photon-in, chemical bond-out” process, a collection of optical imaging and spectroscopy tools with high spatial and temporal resolution must be developed and employed to study this complex semiconductor/bacteria interface. Consequently, we can gain fundamental insights and tease out rate-limiting steps in such energy transduction process across the biotic–abiotic interfaces.

Within the photosynthetic biohybrids, the nonphotosynthetic microorganism performs the critical biological function. For example, the Wood–Ljungdahl pathway (WLP)¹⁴ in acetogenic bacteria is used to reduce CO₂ to acetyl coenzyme A (acetyl-CoA), a common biosynthetic intermediate, and eventually acetic acid. This WLP pathway for acetate synthesis consists of

two separate branches, the methyl-branch and the carbonyl branch (Figure 1). In the carbonyl branch, CO₂ is reduced to CO via the carbon monoxide dehydrogenase/acetyl-CoA synthase (CODH/ACS). In the methyl-branch, the first reaction is CO₂ reduction to formate. The formyl group is further reduced to methenyl-tetrahydrofolate (THF) and then to methyl-THF. The methyl group is eventually transferred via a corrinoid iron–sulfur protein (CoFeSP) to the CODH/ACS. This bifunctional enzyme fuses the bounded CO (from the carbonyl branch) and the methyl group (from the methyl branch) with CoA to form acetyl-CoA.¹⁵ At this point, inert CO₂ is successfully activated and C–C bonds are formed with low energy input through this elegant catalytic network.

The integration of fluorescent semiconductors with bacterial metabolic processes provides a unique optical tag and a noninvasive approach for studying microbial behavior and their metabolic processes. Linking bacteria to an optically addressable semiconductor nanoparticle could aid in spectroscopic studies to elucidate the electron transfer pathway of electrogenic bacteria among other biological responses to abiotic optical, electrical, and chemical stimuli. The first question to ask here is “where do the electrons come from?”. When the solar photons activate the semiconductor nanowires/nanoparticles, the photoexcited electrons will be generated at the semiconductor/electrolyte interface and are fed to the associated microorganisms across the cell membrane. In addition to direct electron transfer, these electrons can also react with water to generate H₂ and reduce redox proteins on the cell membrane.

At the semiconductor/bacteria interface, reducing equivalents will be generated, transported, and can be oxidized by a hydrogenase (HydABC) to generate reduced ferredoxin and NADH, which are in turn used to generate NADPH. NADH, NADPH, and ferredoxin (Fd^{2−}) are all electron carriers in the WLP pathway (Figure 1).¹⁴ In the methyl-branch, the reduction of CO₂ to methyl-THF requires both NADPH and NADH, and the reduced ferredoxin assists in CO₂ reducing to CO. While it is still unclear how those solar-generated electrons that entered from membrane proteins are involved in CO₂ fixation catalytic network, it is believed that they play essential roles as reducing equivalents. Several pathways have been proposed for this initial charge transfer, implicating indirect electron transfer mecha-

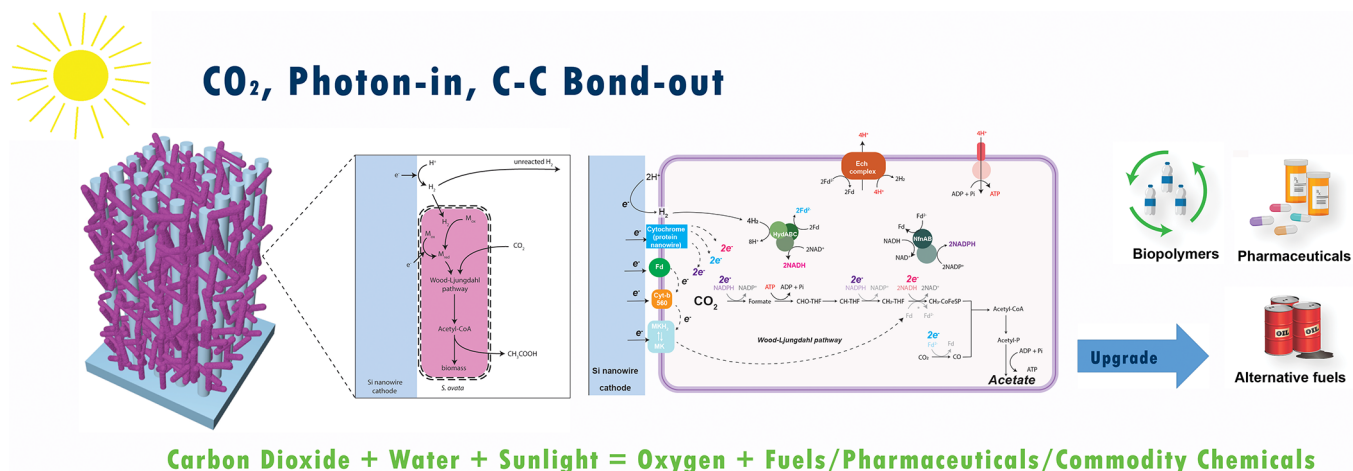


Figure 1. Photosynthetic biohybrids produce liquid sunlight through a “photon-in, chemical bond-out” materials/biology interface that spans multiple orders of magnitude both in the length and time scale. Part of the figure was reprinted from ref⁹ with permission. Copyright 2020, Cell Press.

nisms (via H_2 or uncharacterized soluble redox mediators) or direct mechanisms (unmediated transfer to membrane-bound enzymes, i.e., hydrogenases and cytochromes).

Fundamental to understanding the interface between inorganic and biological components such as CdS and *S. Ovata* is the mechanism of the extracellular electron transfer pathway that accomplishes CO_2 reduction. While the mechanism and integral proteins for electron transfer from bacteria to the metal electrode (as in microbial fuel cells) have been largely elucidated, the basis for cathodic electron transfer in the reverse direction from metal/semiconductor to bacteria remains largely unexplored. Through the determination of the dominant electron pathways, it is possible to gain fundamental insight into this new and novel mode of charge transport and to design more efficient materials or biological systems to exploit these mechanisms for complex molecular synthesis. Studies of the interface between semiconductors and bacteria challenge the research community's current technical and scientific capabilities and signify an unprecedented opportunity toward unraveling the powerful functions from these abiotic/biotic interfaces.

The study of the electron transfer mechanisms at this abiotic–biotic interface represents a significant technical challenge and opportunity. There are three major challenges one needs to overcome: (1) measurement under *in vivo* biological conditions, (2) low sample concentration and signal intensity due to the diffuse nature of biological whole cells, and (3) cell stability and sensitivity to high energy radiation sources. A few techniques meet these criteria, and new imaging and spectroscopic techniques need to be developed to meet these scientific challenges. For example, previously transient absorption spectroscopy (TAS) has been successfully applied to the characterization of hydrogenase–semiconductor nanocrystal systems *in vitro*.¹⁶ Similar experiments are possible on the whole bacterium–semiconductor system. TAS kinetics suggest that the photo-excited reducing equivalents may be taken up by hydrogenase or directly as electrons by membrane-bound proteins.¹⁷ The biochemical activity of proteins involved with charge uptake can be correlated with charge carrier lifetimes through appropriate experimental design.

In addition to kinetic insight, vibrational spectroscopy and X-ray spectroscopy will provide molecular-level insight into these new electron transfer mechanisms. For example, the structure of the Ni–Fe hydrogenase and its spectroscopic signatures throughout its catalytic cycle has been studied *in vitro* through IR, resonance Raman, and X-ray absorption spectroscopy (XAS).¹⁸ However, such techniques have yet to be translated to more complicated whole-cell living systems. Utilizing the light-absorbing semiconductor as an electron source, time-resolved IR spectra can be compared to existing spectroscopic signatures of the Ni–Fe hydrogenase as it proceeds through its catalytic cycle. Greater chemical specificity of the initial steps of electron transfer and downstream metabolic processes can be potentially achieved by using XAS studies. By monitoring the oxidation states of the metal centers of critical metabolic enzymes, the interplay of semiconductor electron transfer and biocatalysis may be analyzed at the initial electron transfer step and throughout the following CO_2 reduction pathway.

In addition to these spectroscopic-based investigations, several research groups are also probing these biohybrids using microbiological methods, including transcriptional and proteomic analyses. Proteomic and metabolomic characterization offers excellent insight into how increased genetic

expression of specific genes activates specific metabolic pathways. For example, Zhang et al. studied these changes in the *M. thermoacetica*–CdS inorganic hybrid system using mass spectrometry and proteomic analysis.¹⁹ The upregulation of electron transfer proteins flavoprotein, ferredoxin, and NADP dehydrogenase suggests the important roles they have in the overall photosynthetic process.

Besides these spectroscopic, transcriptional, and proteomic analyses, one can examine directly how the charge transfer occurs between a microorganism and a solid-state device. We have established a microbial electrochemical cell that comprises a single bacterium interfaced with a single nanowire photo-electrochemical platform.²⁰ The steady-state current transfer from a single electrochemically active bacterium can be directly monitored. Such time-resolved physical contact and the cathodic charge transfer can be correlated with the time-resolved photocurrent measurement under a specific electrochemical bias. With the same experimental platform, one can also apply electrochemical impedance spectroscopy (EIS) as a powerful tool to study charge transfer, mass transport, and the time constant in our nanowire array/bacteria platform. This suite of spectroscopic and photoelectrochemical techniques adapted to conduct measurements on whole cells will shed light on the mechanism of this unique inorganic–biological interface down to the molecular/atomic level. A deep molecular understanding of the interactions involving the semiconductor, the bacterium, and light will further enable a guided search through the vast microbial and enzymatic parameter space to increase the efficiency and performance of such hybrid solar-to-chemical platforms. Simultaneously, these insights will allow the translation of this electron transfer to engineered designer bacteria, expanding the repertoire of the synthetic biology toolbox.

We are setting to advance a new research frontier with these ongoing efforts and establish the body of new knowledge/language for the fundamental understanding of biotic–abiotic interfaces, their electron transfer, and energy transduction mechanism and finally the evolution of the powerful photo-synthetic biohybrids. Through such studies, it is also expected that we will gain a fundamental understanding of the challenging problem of photoactivation of CO_2 , N_2 to produce value-added chemicals, fertilizers, and fuels, such as *n*-butanol, polymer, and other complex natural products, with the energy input purely from sunlight. One could imagine fundamentally in the future that our chemical industry, the energy industry, and pharmaceutical industry can be powered entirely from renewable solar energy rather than mainly relying on traditional fossil fuel. In this way, with liquid sunlight produced from these photosynthetic biohybrids we are providing an ultimate carbon-neutral solution to fundamentally solve the CO_2 emission, global warming, and climate change issues that we are facing on this planet Earth.

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Notes

The author declares no competing financial interest.

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