AgInS$_2$/In$_2$S$_3$ heterostructure sensitization of *Escherichia coli* for sustainable hydrogen production

Zhifeng Jiang$^{a,b,1}$, Bo Wang$^{c,1}$, Jimmy C. Yu$^{c}$, Jianfang Wang$^{d}$, Taicheng An$^{e}$, Huijun Zhao$^{f}$, Huaming Li$^{a}$, Shouqi Yuan$^{e,x}$, Po Keung Wong$^{b,*}$

$^a$ Institute for Energy Research, Jiangsu University, Zhenjiang 21213, China
$^b$ School of Life Sciences, The Chinese University of Hong Kong, Shatin, NT, Hong Kong, China
$^c$ Department of Chemistry, The Chinese University of Hong Kong, Shatin, NT, Hong Kong, China
$^d$ Department of Physics, The Chinese University of Hong Kong, Shatin, NT, Hong Kong, China
$^e$ Institute of Environmental Health and Pollution Control, School of Environmental Science and Engineering, Guangdong University of Technology, 510006 Guangzhou, China
$^f$ Centre for Clean Environment and Energy, Gold Coast Campus, Griffith University, QLD 4222, Australia

**ABSTRACT**

Solar-to-chemical production by photosynthetic biohybrid systems does not only take advantage of the broadband light efficiency of semiconductor but also utilize highly specific biological catalytic power in living organisms. Herein, we demonstrate a tandem inorganic-biological hybrid by combining AgInS$_2$/In$_2$S$_3$ and a facultative anaerobic bacterium, *Escherichia coli*, for biological H$_2$ production. The AgInS$_2$/In$_2$S$_3$@*E. coli* hybrid system harvests light energy and makes use of anaerobically synthesized bacterial endogeneous [Ni-Fe]-hydrogenase and photo-generated electrons from AgInS$_2$/In$_2$S$_3$ hybrid for enhanced H$_2$ evolution efficiency. A highly quantum efficiency (QE) of 3.3% at 720 nm for H$_2$ production is achieved from the hybrid system, exceeding those of many reported photoheterotrophic bacteria. This biomimetic approach may provide a guidance for the interfacing of hybrid semiconductors with living organisms for solar-to-chemical production.

The utilization of renewable and ecologically friendly solar energy is highly desired due to the depletion of energy resources and environment problems [1–3]. Solar-driven hydrogen (H$_2$) evolution has attracted great attention because it provides an ideal strategy by using this clean energy to solve the problem of shortage of finite fossil fuels. Recently, photosynthetic biohybrid systems, which consist of excellent light-absorbing efficiency of inorganic semiconductors and high H$_2$-forming microorganisms, have attracted great attention. Some photosynthetic biohybrid systems for H$_2$ evolution have been exploited where purified hydrogenase and/or genetically engineered microorganisms are employed as biocatalysts [4]. For example, Brown et al. [5] reported a complex of CdS nanorods with purified [Fe-Fe]-hydrogenase, purified from *Clostridium acetobutylicum*, showed good photochemical activity for H$_2$ production. Honda et al. [6] demonstrated that recombinant *E. coli* strain expressed both [Fe-Fe]-hydrogenase and relevant maturases of *C. acetobutylicum* enabled whole cell of bacteria photocatalytically produced H$_2$ with TiO$_2$ in the presence of methyl viologen (MV$^{2+}$) as an electron transfer agent. Nevertheless, the usage of purified hydrogenases from specific microbes, genetic engineered microorganisms and/or MV$^{2+}$ will increase the cost of the strategy, thus may limit its widespread application. An alternative approach for sustainable solar-to-chemical production scheme by photosynthetic biohybrid system is the use of whole bacterial cell without any genetic modification and enzyme purification. Recently, a significant breakthrough work has been accomplished by Sakimoto et al. [7] Their study indicated that bio-precipitation of CdS nanoparticles onto the surface of aceticogenic bacterium *Moorella thermoacetica* can facilitate its photoreductive capability of CO$_2$ to acetic acid. In their following work [8], they proved the inorganic semiconductor-to-bacterium electrons transfer mechanism using spectroscopic elucidation. Their results demonstrated that photoexcited electron transfer can promote the bacterial hydrogenase enzyme activity to generate H$_2$. Inspired by these pioneering studies, we believe that the following two aspects are very important in the construction of photosynthetic biohybrid systems for practical application in the field of solar-to-chemical production: 1) the photo-generated charge carriers should be easily separated, and photo-generated electrons can quickly transfer to the microorganism without the help of electron transfer agents [7]; and 2) the microorganism used...
should be genetic modification free, popular, and can synthesize hydrogenase.

We thus selected an ideal inorganic/whole-cell biohybrid photocatalytic H₂ production system consisting the following two parts: 1) the most well-studied bacterium, *E. coli*, which grows rapidly on a wide range of carbon sources, and 2) a solid-state heterojunction incorporating AgInS₂ with In₂S₃ nanoparticles. *E. coli* can anaerobically synthesize endogenous [Ni-Fe]-hydrogenase without any genetic engineering manipulation [9], and can produce H₂ by dark fermentation. Moreover, it is reported that *E. coli* can produce sulfide to precipitate metal ions and form sulfide complexes on the cell surface under aerobic condition [10]. As an excellent light absorber, the AgInS₂/In₂S₃ junction can afford faster electrical conduction than that of In₂S₃. In this work, In₂S₃ nanoparticles were biologically grown on the surface of *E. coli* by the addition of suitable amount of In³⁺ and cysteine [11,12], and AgInS₂ nanoparticles were anchored on the surface of In₂S₃ via an in-situ ion exchange method under a mild condition (Supporting information-experimental section and Fig. S1). When the hybrid system was irradiated, both AgInS₂ and In₂S₃ could produce photo-generated electrons and holes. The photogenerated electrons on the conduction band (CB) of AgInS₂ would quickly transfer to the CB of In₂S₃, and finally transfer to *E. coli* to carry out microbial H₂ production (Fig. 1). Meanwhile, the photogenerated holes on the valance band (VB) of In₂S₃ would transfer to the VB of AgInS₂, and then oxidize cysteine to cystine (Fig. 1) due to the matched band structure between AgInS₂ and In₂S₃ (Supporting information, Fig. S3) [13–15]. This system can enhance the utilization of photo-generated electrons to participate in the microbial H₂ production by suppressing the recombination of photo-generated electrons and holes (Scheme 1).

High angle annular dark field (HAADF) scanning transmission microscopy (STEM), energy-dispersive X-ray spectroscopy (EDS) mapping and X-ray photoelectron spectroscopy (XPS) were performed to investigate the surface chemical composition and chemical state of the AgInS₂/In₂S₃@*E. coli* hybrid system. From Fig. 1A and B, high densities of bright spots are uniformly dispersed on the surface of *E. coli*. No scattered nanoparticles are observed from the outside of the cell, suggesting the high level interaction between nanoparticles and *E. coli*. High magnification TEM image (Fig. S2) indicates that the average size precipitated nanoparticles is about 15–20 nm. The EDS mapping reveals that the bright spots are composed of In, Ag and S (Fig. 1C-F). Elements of C and N (Fig. 1G and H) are originated from *E. coli* itself. The HAADF-STEM images of thin-sectioned sample indicate that most of the precipitated nanoparticles are located across the periplasmic space of the cell.
bacterial cells. The delicate structure perfectly combines the biological and inorganic system, which can effectively facilitate the electron-transfer within the hybrid system. The full scan survey XPS spectrum of the biohybrid system further confirms the presence of In, Ag, S, O, C, P and N elements (Fig. 1K). The peaks in the high resolution XPS of In 3d (Fig. 1L) at around 444.6 and 451.9 eV correspond to the In 3d5/2 and In 3d3/2, respectively, demonstrating the presence of In 3+ in the AgInS2/In2S3 hybrid system [16,17]. The Ag 3d spectrum (Fig. 1M) consists of two individual peaks at around 368.6 and 374.3 eV, corresponding to Ag 3d5/2 and Ag 3d3/2 binding energies, respectively, which can be attributed to Ag+ [18]. Furthermore, the minor peak of S 2p XPS (Fig. 1N) located at 162.9 eV can be assigned to the sulfur-indium bond, and other peaks may refer to the sulfur present on the E. coli cell surface [17]. XPS results also indicates that the atom ratio of Ag/In is 1/3. As such, all of the above results suggest the successful formation of AgInS2/In2S3 junction.

To demonstrate the applicability of the AgInS2/In2S3@E. coli hybrid system, photocatalytic H2 production experiments were carried out (Supporting information-experimental section). From Fig. 2A, H2 produced from the In2S3@E. coli hybrid system under visible light (> 420 nm, 1400 W m−2) irradiation is 1460 µmol, which is much higher than that produced by E. coli alone. After the formation of AgInS2/In2S3 on the cell surface of E. coli (AgInS2/In2S3@E. coli), the H2 evolution amount (1660 µmol) further increases, which is equivalent to an increase of ~30% as compared to the untreated E. coli culture. During the dark reaction process, it is found that both of In2S3@E. coli and AgInS2/In2S3@E. coli systems show similar amount of H2 produced by E. coli alone (the amount of H2 production by E. coli alone in the dark and visible light irradiation are similar), suggesting the extra H2 produced is ascribed to the effect of precipitated metal sulfide. All these phenomena indicate that the increased H2 production amount by the as-prepared In2S3@E-coli and AgInS2/In2S3@E-coli hybrid systems is...
related to the photocatalytic enhancement by the biologically precipitated single In$_2$S$_3$ nanoparticles and AgInS$_2$/In$_2$S$_3$ inorganic hybrid. According to the previous studies concerning on the heterojunctions [19,20], we may consider the enhanced H$_2$ evolution activity can be attributed the heterojunction electric field between AgInS$_2$ and In$_2$S$_3$, which promotes the photogenerated electron transfer efficiency. The detailed analysis and explanation will be discussed in photochemical section (Fig. 3). As reported by Korinenko et al., [8] upon photo-excitation, the conduction band electrons of the inorganic semiconductor would transfer to membrane-bound protein of microorganism. In order to investigate electron generation and transduction in our system, a series of control experiments were carried out (Fig. 2B, Supporting information) in which 1) E. coli was systematically removed from AgInS$_2$/In$_2$S$_3$@E. coli; 2) equal amount of isolated AgInS$_2$/In$_2$S$_3$ hybrid was mixed with E. coli (separation study); 3) electron scavenger, Cr(VI) was added (electron-scavenger study). In the absence of E. coli, negligible H$_2$ is detected from AgInS$_2$/In$_2$S$_3$ (the heat-treated hybrid system), indicating that the additional H$_2$ is not directly produced from water splitting by the surface AgInS$_2$/In$_2$S$_3$ nanoparticles. No obvious improvement is observed in the H$_2$ production from the separation study in comparison with that produced from E. coli alone, suggests that the close interfacial interaction between the AgInS$_2$/In$_2$S$_3$ hybrid and E. coli is indispensable for the electron transduction. This result is proved by the electron-scavenger study, which shows that the amount of extra H$_2$ decreases after the addition of Cr(VI) to the reaction system. As a result, we can conclude that the semiconductor-to-bacterial cell electron transfer mechanism also works on our system. Specifically, when the biohybrid system is irradiated, the photo-generated electrons on the CB of AgInS$_2$ will first transfer to the CB of In$_2$S$_3$, then the accumulated electrons on the CB of In$_2$S$_3$ will transfer to membrane-bound protein and finally participated in the H$_2$ evolution process [8]. QE of the AgInS$_2$/In$_2$S$_3$ hybrid was also investigated (Fig. 2C, Table S1). The QE of hydrogen production matches well with the UV–Vis absorption spectrum of the AgInS$_2$/In$_2$S$_3$, suggesting that the reaction is driven by light irradiation. We are also delighted to find out that the QE at 720 nm can reach 3.3%, which is higher than many reported photoheterotrophic bacteria [21]. In addition, the H$_2$ evolution without noticeable deactivation was observed even after five cycle tests (Fig. 2D), implying that AgInS$_2$/In$_2$S$_3$@E. coli biohybrid has excellent photo-stability. The cell viability, membrane integrity and genomic DNA content before and after the H$_2$ production process were investigated. No significant stimulation or inhibition of cell viability is observed during the photocatalytic process (Fig. 3A). In fluorescent microscopic study, after staining with a dye mixture, no obvious red fluorescent dye-stained cells can be detected after H$_2$ evolution, further indicating the integrity of the cell membrane (Fig. 3B and C). In addition, from the HAADF image of inset in Fig. 3C, E. coli cell maintains its original structure after the reaction. Moreover, the AgInS$_2$/In$_2$S$_3$ nanoparticles still deposit on the surface of E. coli without falling off. It is also found that malondialdehyde (MDA) [22], a typical lipid membrane peroxidation indicator, does not increase in the solution under aerobic condition (Fig. 3A). Furthermore, the leakage and decomposition of

![Fig. 3. Cell viability and MDA production (A) during the H$_2$ production process, fluorescence microscopic images (B and C, inset: HAADF image of the hybrid after reaction) of E. coli before and after reaction, investigation of leakage and destruction of bacterial genomic DNA extracted from harvested cells during H$_2$ evolution process by means of agarose gel electrophoresis (D) and NanoDrop™ spectrophotometers (E).](image-url)
genomic DNA are not observed from fluorescent intensity of the DNA bands [23]. Also, the DNA concentration remains unchanged during the reaction (Fig. 3D and E). All of the above results suggest that the biohybrid system is relatively active and stable during the H₂ production process under the anaerobic condition.

As aforementioned, the separation-recombination rate of the photogenerated electron-hole pairs is a key factor affecting the efficiency of electron transduction from semiconductor to bacterial because the higher separation rate can enable more electrons to participate in biological H₂ production [24]. To investigate the separation properties of photogenerated electron-hole pairs, a series of photoelectrochemical tests were carried out. As a photocathode, the photocurrent density of AgInS₂/In₂S₃ hybrid is consistently higher than that of In₂S₃ (Fig. 4A). Nyquist plot (Fig. 4B) obtained from AgInS₂/In₂S₃ hybrid exhibits a smaller arc radius as compared with In₂S₃, confirming the higher electronic conductivity of AgInS₂/In₂S₃ hybrid due to the effective

Fig. 4. Linear sweeps voltammograms (A) of In₂S₃ and AgInS₂/In₂S₃ (without E. coli) inset: transition photocurrent at 0.4 V versus Ag/AgCl, nyquist plots (B) of In₂S₃ and AgInS₂/In₂S₃ in 0.1 M Na₂SO₄ solution under dark and visible light, open circuit potential (C) of In₂S₃ and AgInS₂/In₂S₃, inset: normalized open-circuit potential decay curves, steady state photoluminescence (PL) spectra (D) of In₂S₃ and AgInS₂/In₂S₃ at excitation wavelength of 438 nm.
The average recombination rate can be calculated by the following:

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The combination rate of AgInS2/In2S3 is e.

When AgInS2/In2S3 hybrid are irradiated with visible light, the arc radiuses are decreased, indicating that the electronic conductivities increased under light irradiation. The surface recombination rate was further studied using the decay profile of open circuit potential (Voc) (Fig. 4C).

The average recombination rate can be calculated by the following equation: \((V_{\text{oc}})/k = 1-\exp(-kt)\), where \(V\), \(V_{\text{dark}}\), and \(V_{\text{light}}\) are the \(V_{\text{oc}}\) at any time, in the dark and under light irradiation; \(k\) is the pseudo-first order recombination rate constant [25]. AgInS2/In2S3 (0.084 s\(^{-1}\)) shows slower decay kinetic in comparison with In2S3 (0.097 s\(^{-1}\)) after turn-off the light, demonstrating that the surface recombination rate of AgInS2/In2S3 is effectively suppressed as compared with In2S3. Moreover, AgInS2/In2S3 hybrid also shows significantly reduced PL intensity, relative to In2S3, indicating a more efficient electron-hole separation on the as-prepared hybrid (Fig. 4D).

To investigate pivotal factors affecting \(H_2\) production performance, a series of experiments were carried out. In the biological \(H_2\) evolution process of AgInS2/In2S3@E. coli system, the available intracellular glucose serves as the original energy source and electron donor. Glucose is quickly consumed within 1 h both for dark control and visible light irradiation (Fig. 5A), and no obvious difference can be detected, suggesting that the glucose can be rapidly absorbed by E. coli and quickly involved in the glycolysis process. In addition, compared to the dark control group, a lower lactate fermentation activity is exhibited in the light irradiation group during the 3 h reaction (Fig. 5B), indicating that the unfavourable pathway in fermentative \(H_2\) production is inhibited by the photo-generated electrons, and more energy sources flowed into the formic acid and \(H_2\) production pathway. In contrast, a significantly higher intracellular formate concentration in the light irradiated hybrid cells is observed (Fig. 5C). The result illustrates that more substrates for \(H_2\) generation are accumulated under visible light. In addition, the stimulating effect on the FDH activity by the photo-generated electrons is also remarkable (Fig. 5D), resulting in a stronger catalytic power for \(H_2\) production. Higher intracellular reduction potential under light irradiation is also detected from the NADH/NAD ratio test (Fig. 5E), illustrating that the hybrid cells are under higher stress of reducing potential and have to dispense with excess reducing equivalents to maintain the redox balance. The more accumulated NADH in the irradiated group also corresponds to the inhibited lactate fermentation in this experiment. It is therefore reasonable that with the help from photogenerated electrons from cell surface AgInS2/In2S3, the hybrid cells can obtain more energy and higher catalytic activity for the fermentative \(H_2\) production pathway, which finally lead to the enhanced biological \(H_2\) production.

To conclude, the \(H_2\) production from solar-driven (bio)photocatalysis has great potential to be a green technology to address global energy shortage and environmental pollution problems. In our present work, we developed a biohybrid system containing the most well-studied bacterium, E. coli and its biologically precipitated AgInS2/In2S3 hybrid. This system has the advantages of genetic manipulation free, using no electron transfer agents and high efficient electronic transmission. This is also the first report to make use of AgInS2/In2S3 hybrid in the biohybrid system for conducting \(H_2\) production. This strategy could be expanded to other metal sulfide hybrids as inorganic light harvesters to construct biohybrid system for utilization of solar energy.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.nanoen.2018.02.001.

References

Zhifeng Jiang obtained BS degree (2010) in Chemistry and Ph.D. degree (2016) in Environmental Science from Jiangsu University. He studied Surface Science under the guidance of Professor Jingguang Chen as a joint Ph.D. student in Columbia University (2015–2016). By the support of Hong Kong Scholars Program, he joined the Professor Po Keung Wong’s group in School of Life Sciences of The Chinese University of Hong Kong. His research interests include photocatalysis (photocatalytic water splitting and CO2 reduction) using traditional semiconductors as well as organic-biological biohybrid systems and hydrodeoxygenation of oxygen-containing small molecules with surface science study and DFT calculation.

Bo Wang obtained his BS degree from Hubei Normal University (2011), MS degree from Tsinghua University (2014), and Ph.D. degree from School of Life Science, The Chinese University of Hong Kong (2017). He joined in Department of Chemistry of The Chinese University of Hong Kong as a research assistant in 2017. His current research focuses on constructing inorganic-biological hybrid system for energy production and photocatalytic disinfection of microorganism.

Jimmy C. Yu is Choh-Ming Li Professor of Chemistry at The Chinese University of Hong Kong. He graduated from St. Martin’s College in 1980, and received a Ph.D. from the University of Idaho in 1985. He joined the Department of Chemistry at CUHK in 1995 as an associate professor, and was promoted to professor in 2002. Professor Yu is a leading scientist in the field of photocatalysis. He is a prolific writer who also holds several patents on photocatalytic nanomaterials. Professor Yu is now Head of United College and Associate Director of the Institute of Environment, Energy and Sustainability.

Jianfang Wang obtained BS degree from University of Science and Technology of China (1993), MS degree from Peking University (1996), and Ph.D. degree from Harvard University (2002). He did postdoctoral study in University of California, Santa Barbara (2002–2005). He joined in Department of Physics of The Chinese University of Hong Kong as an assistant professor in 2005 and became an associate professor in 2011 and full professor in 2015. His current research focuses on nanophotonics and photocatalysis. He has published ~170 papers, with a total citations of ~17,620 (Google Scholar: ~22900) and an h-index of 58 (Google Scholar: 63).

Taicheng An received his Ph.D. degree from Sun Yat-Sen University in 2002, and currently is a professor and the director of Institute of Environmental Health and Pollution Control, Guangdong University of Technology, China. He has awarded over 60 patents (40 issued) and published over 240 referred SCI papers. He was selected as one of the most cited Chinese authors in Environmental Sciences by Elsevier’s Scopus from 2014 to 2016. He is a NSFC Distinguished Young Scholar, Distinguished Professor of the Pearl River Scholars Program, and Young Scientist Winner of Scientific Committee on Problems of the Environment in China.

Huijun Zhao is a Professor and the Direct of the Centre for Clean Environment and Energy at Griffith University, Australia. He is also the Director of the Centre for Environmental and Energy Nanomaterials, The Institute of Solid State Physics, Chinese Academy of Sciences. He has expertise in energy and environmental materials, water source control and management system, field-based sensing technologies and aquatic environment quality assessment. One of his current pursuits is to explore new means to unlock the catalytic powers of nonprecious materials as high performance catalysts for important catalysis reactions.

Huaming Li received his BS degree from China West Normal University in 1985 and master degree from the Chinese Academy of Sciences in 1992. At present, he is a full professor at the Jiangian University. His current research is focused on nanomaterials, ionic liquids for energy and environmental applications. He is the author and co-author of 340 original research papers published in SCI journals. The total number of citations for his work has reached about 8600 and his H-index is 52.

Shouqi Yuan is currently a full professor and Director of Institute for Energy Research, Jiangsu University. He received his Ph.D. degree from Jiangsu University in 1994. He is the editor-in-chief of Journal of Jiangsu University, Vice President of Asian Agricultural Engineering Association, Executive Director of China’s Agricultural Engineering Association. His current research interests include energy conservation technology and energy application.

Po Keung Wong has 40 years research experience on environmental science and technology. He received his B.Sc. and M.Phil. degrees from The Chinese University of Hong Kong (CUHK) and Ph.D. degree from University of California, Davis. He returned to CUHK to start his professorial career in 1986. Currently he is a professor in School of Life Sciences and the Associate Director of Environmental Science Programme. He also is visiting professor for Nankai University, South China University of Technology, Central China Normal University and Guangdong University of Technology in China. His current research focuses on photocatalytic water purification and energy production.