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Photocatalysis

Enhanced Light-Driven Hydrogen Production by Self-Photosensitized Biohybrid Systems

Mónica Martins, Catarina Toste, and Inês A. C. Pereira**

Abstract: *Storage of solar energy as hydrogen provides a platform towards decarbonizing our economy. One emerging strategy for the production of solar fuels is to use photocatalytic biohybrid systems that combine the high catalytic activity of non-photosynthetic microorganisms with the high light-harvesting efficiency of metal semiconductor nanoparticles. However, few such systems have been tested for H_2 production. We investigated light-driven H_2 production by three novel organisms, Desulfovibrio desulfuricans, Citrobacter freundii, and Shewanella oneidensis, self-photosensitized with cadmium sulfide nanoparticles, and compared their performance to Escherichia coli. All biohybrid systems produced H_2 from light, with D. desulfuricans-CdS demonstrating the best activity overall and outperforming the other microbial systems even in the absence of a mediator. With this system, H_2 was continuously produced for more than 10 days with a specific rate of 36 $\mu mol\, g_{dcw}^{-1}\, h^{-1}$. High apparent quantum yields of 23 % and 4 % were obtained, with and without methyl viologen, respectively, exceeding values previously reported.*

Introduction

The use of hydrogen as an energy vehicle has seen a renewed interest in recent years driven by the increasing global commitment to contain climate change, improvements in technology, increase in hydrogen and fuel cell commercialization and the idea of adopting existing infrastructure to facilitate the transition towards using hydrogen. Hydrogen will help to decarbonize multiple areas, such as heating, transport, industry and energy storage.[1] Hydrogen production is still based mainly on fossil fuels, but water electrolysis from renewable electricity is gaining ground. However, this technology relies mostly on rare and precious metal catalysts, limiting its sustainability and scale-up potential.[2] Thus, the quest for novel H_2 production technologies continues, especially those based on direct conversion of solar energy.

Current photovoltaic technology based on inorganic semiconductors has largely surpassed natural photosynthesis in terms of solar conversion efficiencies ($\approx$20 % for conversion to electricity vs. $\approx$3 % for end products of photosynthesis), but similar high conversion efficiencies have still not been achieved for converting light energy into chemical bonds. An emerging attractive technology that tries to solve this conundrum is based on semi-artificial photosynthesis, which combines the power of synthetic catalysts for harvesting light energy with the unsurpassed efficiency and specificity of biological catalysts for chemical reactions.[3–6] This technology involves the development of photosynthetic biohybrid systems, where the biological components (enzymes or microorganisms) are coupled with synthetic light-harvesting materials. A lot of work has been devoted to investigating the coupling of semiconducting materials with enzymes,[3,6–8] namely with hydrogenases for photocatalytic hydrogen production,[6,9–12] but the inherent instability of the proteins and the costs associated with purification and manipulation restrict their commercial application. To overcome these limitations, biohybrid systems have recently started to be developed using microorganisms as catalysts, as these can comprise complex biosynthetic pathways, are far more stable than isolated proteins and have the power for self-renewal and reproduction.[3–6] This approach allows for the use of non-photosynthetic microorganisms that can harbor pathways for more elaborate products than photosynthetic ones, and/or present higher catalytic efficiencies. It is also especially appealing when the microbes are coupled with light-harvesting nanoparticles or nanostructured materials due to their superior optical and electronic properties, high surface area and ease of interaction due to similar dimensions. Initial studies in this area explored organisms such as *Clostridium butyricum* and *Escherichia coli* for photocatalytic H_2 production using a redox mediator that transferred electrons between semiconductor particles, such as TiO_2 and Bi_2O_3, and the organisms.[13–16] A similar approach recently explored *Shewanella oneidensis* and water-soluble photosensitizers to produce H_2 and reduce pyruvate, fumarate and CO_2 to formate.[17] However, this methodology does not involve direct interaction between the cells and the light harvesting materials, requiring electron transfer through toxic and expensive redox mediators, which also limits the catalytic efficiency. In addition, these systems integrate chemically-produced semiconductors, whose synthesis often requires complex and energy-intensive techniques.

A recently developed, more elegant strategy, involves the use of biohybrid systems constructed by photosensitizing non-photosynthetic microbes with self-produced metal semiconductor nanoparticles.[18,19] Sakimoto and colleagues pioneered this approach in a landmark study where they used the highly efficient light harvesting capacity of cadmium sulfide biosynthesized by *Moorella thermoacetica* to produce acetate from CO_2, using cysteine as sacrificial electron donor.[18] In this

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system, the CdS nanoparticles self-precipitated on the cell surface of *M. thermoacetica* deliver electrons to intracellular pathways for CO_2 reduction. The mechanism involved was further probed with spectroscopic, proteomic and metabolomic techniques, suggesting involvement of hydrogenases, energy-related membrane proteins and enzymes of the acetyl-CoA pathway and tricarboxylic acid cycle.[20,21] Inspired by this work other CdS biohybrid systems have recently been developed, namely for light-driven CO_2 reduction.[3–6,22–24] Nevertheless, the field of photosensitizing microorganisms is still in its infancy and many avenues need to be further investigated, namely by testing a wider range of microbes and light-harvesting materials, targeting different pathways and products and exploring the power of synthetic biology for microbial pathway engineering. For example, the self-photosensitizing approach that allows direct interaction between microorganisms and nanoparticles has been poorly investigated for light-driven H_2 production, and has only been tested with *E. coli* and CdS or $AgInS_2/In_2S_3$ semiconductors.[25–27] In these studies H_2 production required glucose or other organic nutrients as electron donors, and modest ≈30% increases in H_2 production were observed, relative to the absence of light.

We proposed to investigate if the self-photosensitization approach could be more efficiently applied for H_2 production using microorganisms that are known to express high levels of hydrogenases and/or are efficient in producing sulfide or in electron exchange with external materials. In this work, three new biohybrid systems were generated using Gram-negative bacteria and their self-precipitated CdS nanoparticles, for H_2 production from light using cysteine as sacrificial electron donor (Figure 1). *Desulfovibrio desulfuricans* was chosen as a member of the sulfate reducing bacteria that have high activity for H_2 production,[28–30] and have the additional advantage of generating sulfide as major metabolic product from sulfate respiration, making them very efficient in the self-production of metal nanoparticles.[31,32] *D. desulfuricans* was previously shown to be highly active in H_2 production.[33] *Citrobacter freundii* was chosen as another sulfide and H_2-producing bacterium,[34] *S. oneidensis* as a H_2-producing electroactive microorganism efficient in electron exchange with inorganic materials[35,36] and the model *E. coli* for comparison with previous studies.

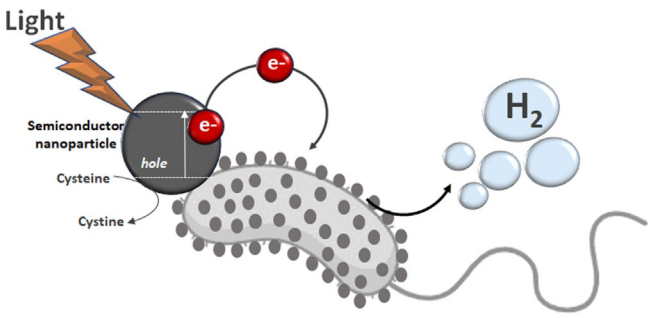

Figure 1. Schematic representation of light-driven hydrogen production by the biohybrid systems composed by bacterial cells and self-produced CdS nanoparticles.

Results and Discussion

Cadmium sulfide is one of the most prominent semiconductors due to its excellent photocatalytic properties and narrow band gap (2.39 eV), making it an attractive visible-light harvesting material.[37,38] As an alternative to the expensive and environmentally-unfriendly chemical methods, CdS nanoparticles can be biosynthesized by several microorganisms through the reaction of cadmium with hydrogen sulfide produced biologically by assimilatory or dissimilatory pathways.[39,40] These biogenic CdS nanoparticles are normally attached to the cell surface creating a unique interaction between metal nanoparticles and microorganism that allows for direct electron transfer within the biohybrid systems.

Characterization of the biohybrid systems

For generation of the biohybrid systems, cells grown in the presence of a sulfur compound (sulfate for *D. desulfuricans*, cysteine and thiosulfate for *S. oneidensis* and only cysteine for *C. freundii* and *E. coli*) were incubated with $CdCl_2$. The effective synthesis of the biohybrids could be observed by development of a yellow color, indicative of CdS formation, and resulted in full removal of cadmium from solution. The biohybrid systems were characterized by SEM and SEM-EDS (Figure 2). The SEM images revealed the presence of nanoparticles on the cell surface of all bacteria (Figure 2 B/C, E/F, H/I, and K/L), whereas in control cells no particles were observed (Figure 2 A, D, G and J). SEM-EDS showed that the nanoparticles were mainly composed by cadmium and sulfur (Supporting information, Figure S1), confirming the precipitation of cadmium as cadmium sulfide. The metals used in sample preparation (Os, Ag and Pd) were also detected.

The SEM images showed a remarkable high density of CdS nanoparticles on the surface of *D. desulfuricans*, along with some extracellular CdS nanoparticle clusters (Figure 2 B/C). In the case of *C. freundii* mostly extracellular clusters of CdS nanoparticles were observed around of the cells (Figure 2 E/F). The CdS nanoparticles formed by *S. oneidensis* appeared as smaller spherical spots uniformly distributed on the cell surface (Figure 2 I/H), in agreement with previous studies.[41,42] In the case of *E. coli*, small clusters of CdS particles were observed on the cell surface (Figure 2 K/L). The biohybrid systems were further characterized by X-ray powder diffraction (XRD, Figure 3). For all four cases the diffraction patterns confirmed the crystalline nature of the CdS particles produced, with XRD patterns consistent with that of crystalline hexagonal CdS (standard card JCPDS-00-041-1049). The significant peak broadening observed relative to the standard agrees with the small size of the CdS nanoparticles.[43] The *E. coli* diffraction pattern reveals a more amorphous nature of this sample, which is probably caused by its higher cell density.

The UV/Vis absorption spectra and Tauc plots of the biohybrid systems revealed direct band gaps of 2.44, 2.58, 2.64 and 2.41 eV, for *D. desulfuricans*, *C. freundii*, *S. oneidensis* and *E. coli* CdS systems, respectively (Figure 4). The larger measured band gaps relative to bulk CdS (≈2.39 eV) reveal

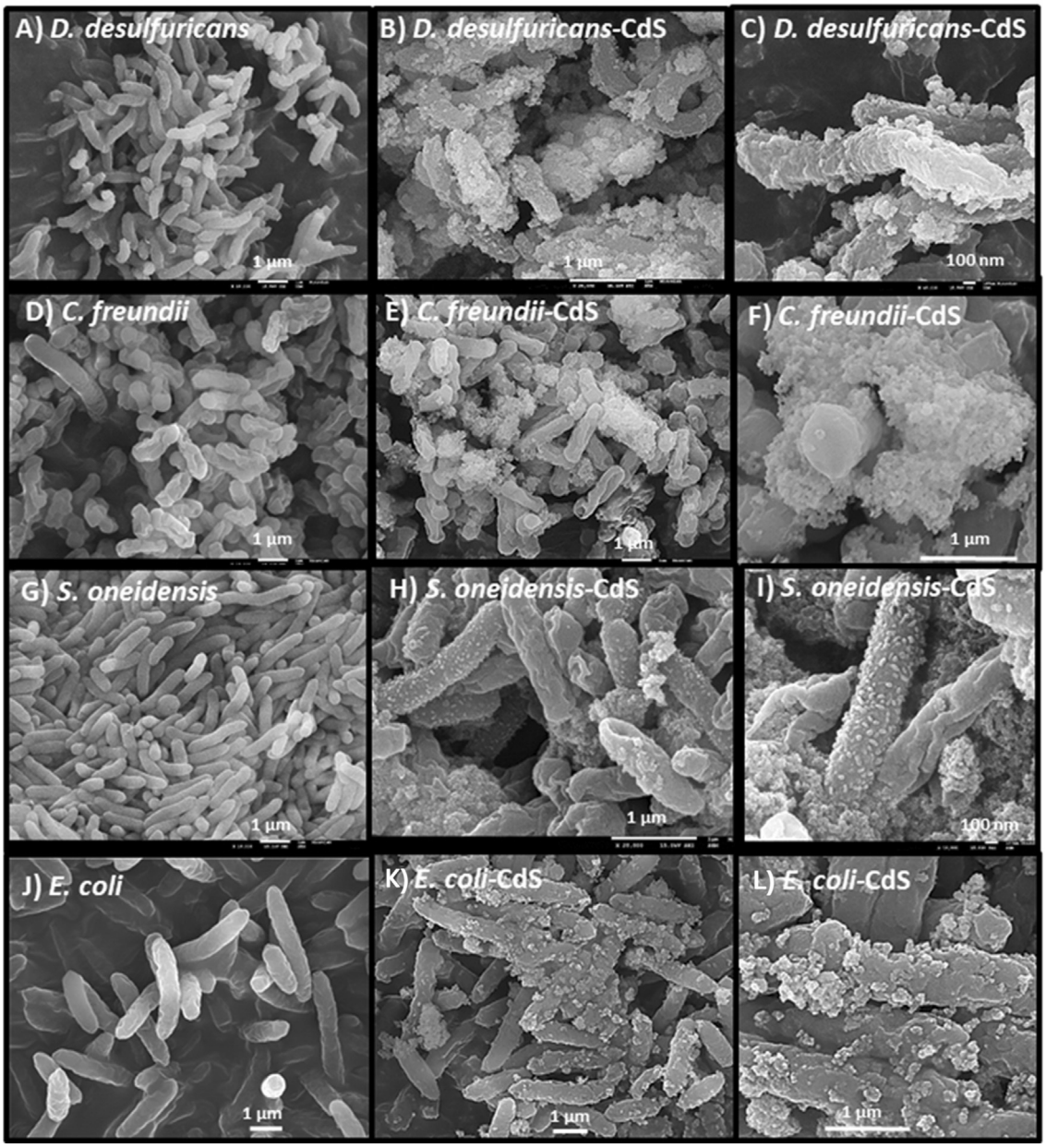

Figure 2. Electron microscopy analysis of the biohybrid systems. SEM images of isolated pre-treated cells: A) *D. desulfuricans*, D) *C. freundii*, G) *S. oneidensis* and J) *E. coli*), and CdS biohybrid systems: B,C) *D. desulfuricans*, E,F) *C. freundii*, H,I) *S. oneidensis* and K,L) *E. coli*).

a quantum confinement effect and indicate that smaller particles are produced by *S. oneidensis*, as observed by SEM, followed by *C. freundii*, *D. desulfuricans* and *E. coli*.[43,44]

Hydrogen production profile

The biohybrid systems were irradiated with light in a cysteine-containing solution in the presence or absence of methyl viologen (MV). To enable a direct comparison between the different systems, the specific H_2 production rate (per g of dry cell weight) is reported, taking into account the amount of cells used in each experiment. All biohybrid systems were able to produce H_2 from light, albeit with different magnitudes (Figure 5). In the presence of MV, the production of H_2 by the *D. desulfuricans*-CdS biohybrid was very high at 10800 µmol g_{dcw}^{-1} after 120 h. This is considerably higher than that of the other three organisms, with the *S. oneidensis*-CdS biohybrid producing 2000 µmol g_{dcw}^{-1}, the *C. freundii*-CdS system 858 µmol g_{dcw}^{-1} and the *E. coli*-CdS system 1200 µmol g_{dcw}^{-1}, after 140 h (Figure 5a). In the absence of MV, the H_2 production of the biohybrid systems was reduced between 30 to 40% for all organisms (Figure 5b), with the notable exception of the *S. oneidensis*-CdS system, which showed identical performance in the presence and in the absence of MV (Supporting information Figure S2). Remarkably, the *D. desulfuricans*-CdS biohybrid without MV outperformed the other systems

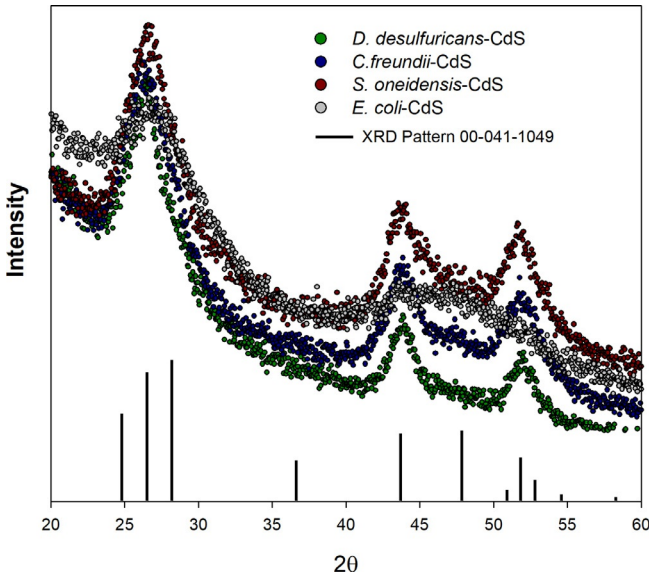

Figure 3. X-Ray diffraction patterns of the CdS biohybrid systems.

(3790 μmol g_{dcw}^{-1} of H_2), even when these were run in the presence of MV. In contrast, *E. coli* displayed a modest activity in the absence of glucose.

Several control experiments were carried out (Supporting information Figure S2) and no production of H_2 was detectable in the absence of light, or with regular cells incubated with MV and irradiated with light. With heat-treated biohybrid systems, where the biological catalysts are inactivated, only a very low production of H_2 was observed (123, 67, 397 and 150 μmol g_{dcw}^{-1} by *D. desulfuricans*-CdS, *C. freundii*-CdS, *S. oneidensis*-CdS and *E. coli*-CdS, respectively), demonstrating that the chemical activity of the CdS nanoparticles *per se* is very low compared to the complete biohybrids. These control experiments prove that H_2 is produced mainly by the cells and only under illumination.

The behavior of the *S. oneidensis*-CdS system is unusual, as a higher photocatalytic activity is usually observed in the presence of an electron shuttler.[15–17,27] *S. oneidensis* is a well-known electroactive microorganism able to perform extracellular electron transfer with insoluble electron acceptors/donors directly and indirectly.[35,36] In direct electron transfer this bacterium establishes contact via proteins that decorate the cell surface, like MtrC and OmcA cytochromes, or through cellular appendages like conductive nanowires. On the other hand, indirect electron transfer occurs through redox active compounds produced by *S. oneidensis*, such as flavins.[35,36] In fact, it has been reported that flavin electron shuttling is responsible for up to 75% of extracellular electron

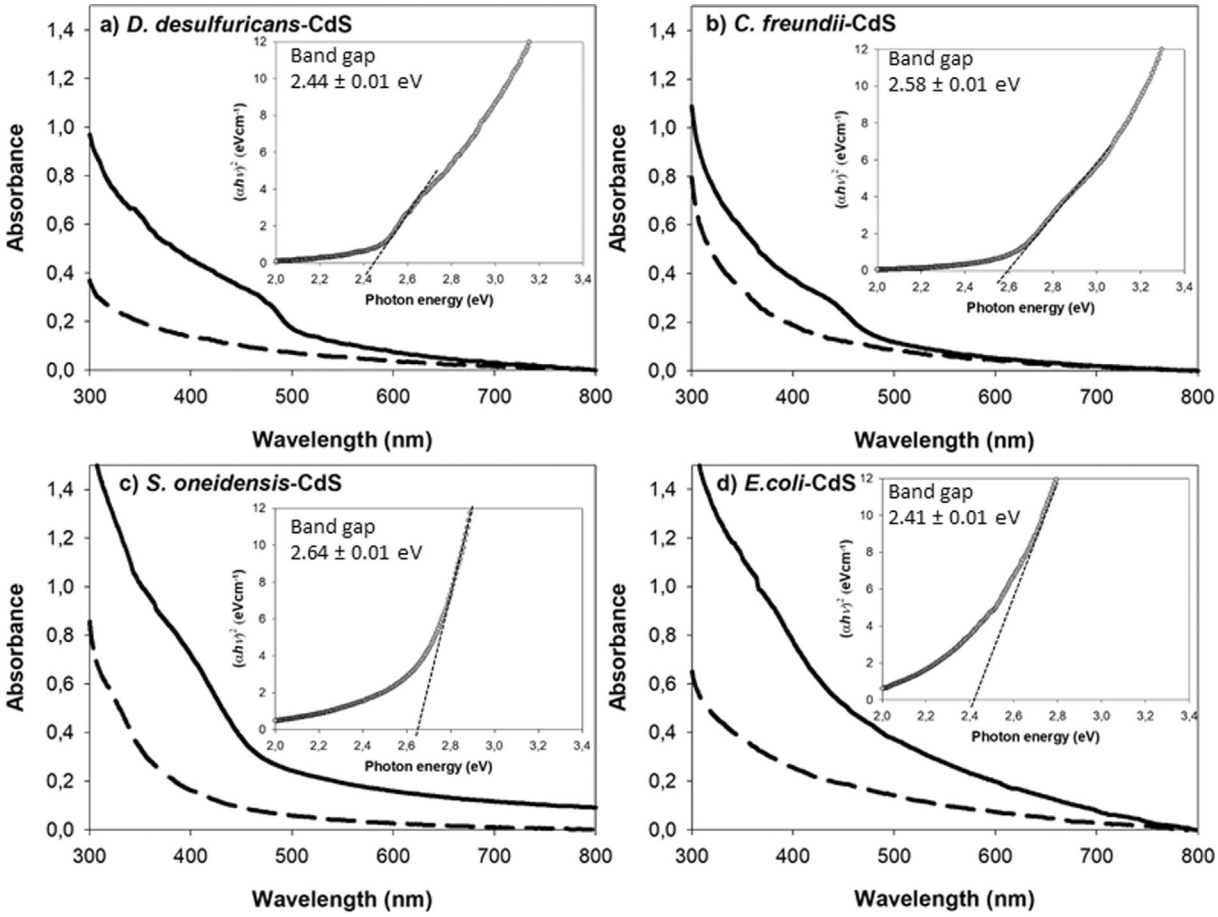

Figure 4. UV/Vis absorption spectra and Tauc Plots (inserts) of the CdS biohybrid systems. Solid and dashed lines represent the cells-CdS and cells spectra, respectively.

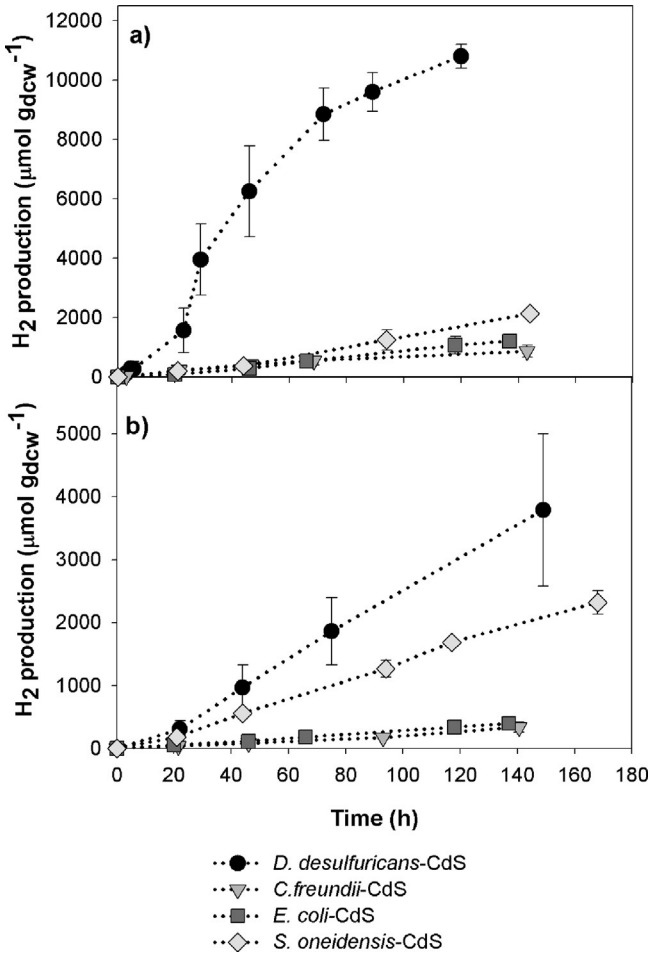

Figure 5. Hydrogen production profile of biohybrid systems in the presence (a) and absence (b) of methyl viologen (MV) as redox mediator. All systems were generated using 1 mM Cd. Error bars indicate the standard deviations of three independent experiments.

transfer by *S. oneidensis*.[45] These mechanisms, and in particular flavin electron shuttling, may be responsible for the ability of the *S. oneidensis*-CdS system to operate similarly in the presence or absence of MV. In contrast, Rowe and colleagues reported a requirement for MV in light-driven H_2 production by a photocatalytic system composed by *S. oneidensis* and the chemical photosensitizer eosin Y.[17]

Overall, the most efficient biohybrid system was the one obtained with *D. desulfuricans*. Since the total amount of CdS and its crystalline structure is comparable in the four systems, and given that the *D. desulfuricans*-CdS biohybrid presents a lower band gap than *C. freundii*-CdS or *S. oneidensis*-CdS, this suggests that its higher H_2 production might be related with a higher biological activity and/or more efficient electron transfer with CdS, rather than the intrinsic properties of the nanoparticles produced by this organism. The hydrogenase activity of all four organisms was determined using reduced methyl viologen as electron donor (Table 1). The *D. desulfuricans* cells showed the highest hydrogenase activity followed by *S. oneidensis*, *E. coli* and *C. freundii* with the values of 280, 8.4, 3.7 and 1.6 $\mu mol\, g_{dcw}^{-1}\, min^{-1}$, respectively, which shows a good correlation (albeit on a different time scale) with the relative values of the photosynthetic H_2 production

Table 1: Hydrogen production rates of whole cells (from dithionite-reduced MV) and the biohybrid systems (from light).

Microorganism	Whole cells [$\mu mol\, g_{dcw}^{-1}\, min^{-1}$] + MV	Biohybrid systems + light [$\mu mol\, g_{dcw}^{-1}\, h^{-1}$] + MV
D. desulfuricans	280 ± 9	130 ± 8
C. freundii	1.6 ± 0.1	8.0 ± 0.7
S. oneidensis	8.4 ± 0.5	14.4 ± 0.9
E. coli	3.7 ± 0.1	9.3 ± 0.7

rates obtained with the biohybrid systems. *D. desulfuricans*, as other sulfate reducing bacteria, is characterized by a high level of hydrogenases belonging to the [FeFe] and [NiFe] families,[28] most of which are present in the periplasm and are thus likely to be more efficient in receiving electrons directly from CdS nanoparticles than intracellular hydrogenases. This microorganism contains three periplasmic hydrogenases, the soluble [FeFe] HydAB, [NiFe] HynAB, and HynABC, and two membrane-bound [NiFe] Ech and Coo hydrogenases.[28] To further evaluate the role of hydrogenases, H_2 production by the *D. desulfuricans*-CdS system was studied in the presence of cyanide, a well-known inhibitor of these enzymes.[46] Cyanide caused a nearly complete inhibition of H_2 production by the biohybrid system (98%, Supporting information Figure S3), resulting in an activity level (223 $\mu mol\, g_{dcw}^{-1}$ of H_2) similar to the heat-inactivated system.

Optimization of light-driven H_2 production by the D. desulfuricans-*CdS biohybrid*

To further enhance the photocatalytic activity of the *D. desulfuricans*-CdS system, we tested biohybrids produced under different cadmium concentrations and cell loads. A low Cd concentration (< 1 mM) resulted in reduced light harvesting efficiency, while Cd concentrations higher than 3 mM decreased the activity of the biohybrids, probably due to toxicity (Figure 6a). In the case of the *Methanosarcina barkery*-CdS and *M. thermoacetica*-CdS biohybrids, the highest photocatalytic activities for CO_2 reduction were observed with 1 mM Cd.[18,23] In contrast, for the *D. desulfuricans*-CdS system the highest activities were observed with 2 or 3 mM of Cd, reaching 20 μmol H_2 after 24 h of light irradiation in a small scale experiment. In terms of cell load the best activity was observed with 5.3 mg_{dcw}, where the maximum value of 30 μmol H_2 was attained (Figure 6b). Further increasing the number of cells did not resulted in an increase of H_2 production.

The light-driven H_2 production of the *D. desulfuricans*-CdS biohybrid system generated in the optimal conditions (3 mM Cd and cell load of 5.3 mg_{dcw}) was investigated under two different light sources (Figure 7).

Under LED illumination ($\lambda = 445$ nm and an irradiance of 0.042 $mW\, cm^{-2}$), and in the presence of MV, H_2 production reached the maximum of 55 μmol (Figure 7a). Under these conditions, H_2 was produced with an initial specific rate of 418 $\mu mol\, g_{dcw}^{-1}\, h^{-1}$. Without MV, H_2 was continuously produced for more than 240 h with an initial specific rate of

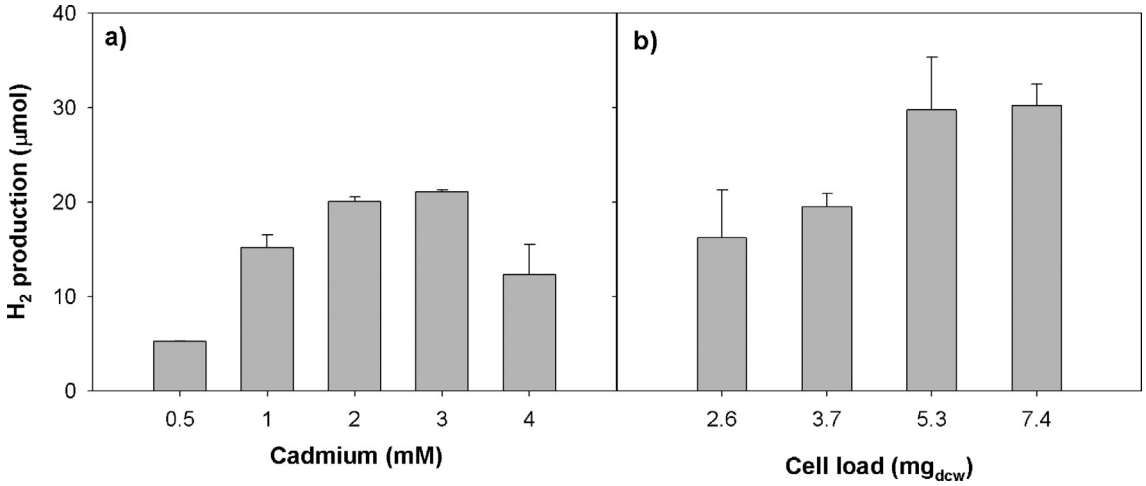

Figure 6. Effect of cadmium concentration (a) and cell load (b) on the performance of the *D. desulfuricans*-CdS hybrid system. The effect of cadmium concentration was evaluated using 3.6 mg_{dcw} of *D. desulfuricans*, while the effect of cell load was evaluated using the *D. desulfuricans*-CdS synthesized with 3 mM Cd. The experiments were carried out with 6.5 mL of working volume in the presence of methyl viologen. The data are for 24 h of light irradiation and error bars indicate the standard deviations of three independent experiments.

36 μmol g_{dcw}^{-1} h^{-1}. After 240 h of light irradiation, 37 μmol of H_2 had been produced. Under high light intensity, the H_2 production rate increased substantially. The *D. desulfuricans*-CdS biohybrid illuminated with a Xenon lamp (21 W cm^{-2}) was able to produce H_2 with a rate of 1057 and 827 μmol g_{dcw}^{-1} h^{-1}, in the presence and absence of MV, respectively (Figure 7b). These rates are 2.5 and 23-fold higher than the ones observed with the violet LED with and without MV, respectively. After 5 hours of Xenon lamp irradiance the system produced 28 and 21 μmol of H_2 with and without MV respectively.

Under LED light, in the presence of MV, the system began to plateau after 45 h of irradiation (Figure 7a), which could be related with depletion of the sacrificial electron donor. To investigate this, the H_2 production by *D. desulfuricans*-CdS biohybrid system was evaluated with different amounts of cysteine (Figure 8).

In the absence of sacrificial electron donor to quench the photogenerated holes the system only produced 5 μmol of H_2. The H_2 production increased with increasing amount of cysteine, as previously observed.[23,47] After 44 h of light irradiation, the H_2 production increased from 35 μmoles with 60 μmol of cysteine to 80 μmoles with 180 μmol of cysteine, corresponding to a hydrogen yield of 117% and 89% based on the cysteine added [Eq. (S1) and (S2) in Supporting information]. The H_2 yield higher than 100% is explained by the small amount (5 μmol) produced without sacrificial electron donor. These results confirm that the cessation of H_2 production observed was caused by cysteine depletion.

The apparent quantum yield (AQY) of the biohybrid system under LED illumination ($\lambda = 445$ nm and an irradiance of 0.042 mW cm^{-2}) was determined assuming that all the emitted light was harvested by the system, which underestimates the AQY [Eq. (S3) in Supporting information]. An AQY of 23% and 4% was achieved, with and without MV, respectively, which is higher than that reported for most biohybrid systems with self-produced semiconductor nano-

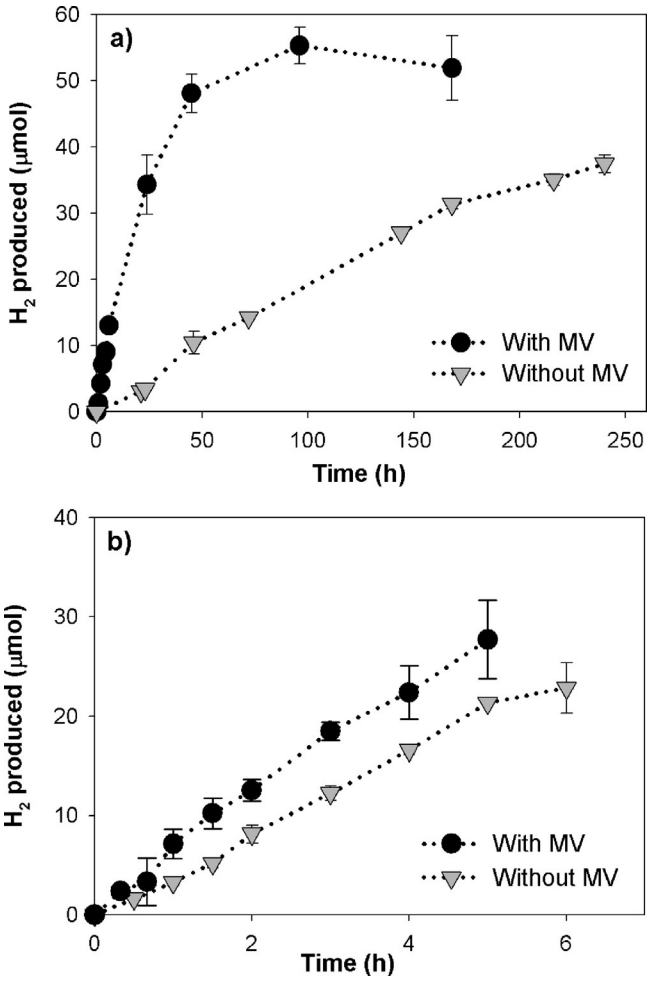

Figure 7. Light-driven H_2 production by the *D. desulfuricans*-CdS biohybrid system under two light sources: LED (a) and Xenon lamp (b), and 100 μmoles of cysteine. The biohybrid system was generated under the best conditions (3 mM cadmium and 5.3 mg_{dcw}). The experiments were carried out with 6.5 mL of working volume. Error bars indicate the standard deviations of three independent experiments.

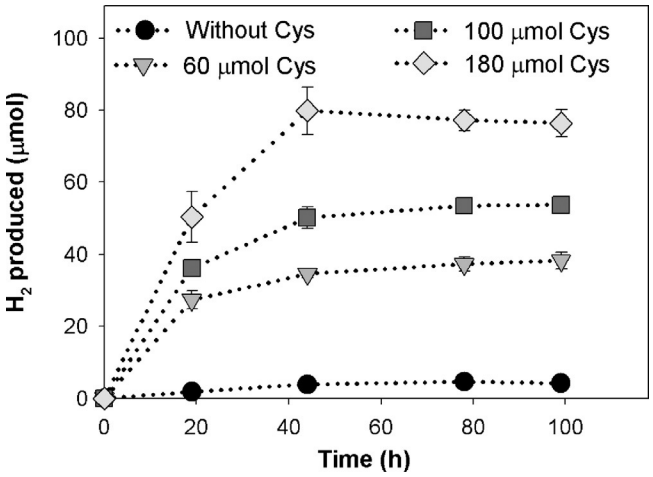

Figure 8. Effect of cysteine on light-driven H_2 production by the *D. desulfuricans*-CdS biohybrid system. The biohybrid system was constructed under the best conditions (3 mM cadmium and 5.3 mg_{dcw}) and irradiated with LED light. The assays were carried out with 6.5 mL of working volume in the presence of MV. Error bars indicate the standard deviations of three independent experiments.

particles.[18,23,25,26,47] Moreover, the system demonstrated a remarkable stability. In the absence of MV the system produced H_2 continuously for more than 10 days, while in the presence of the electron shuttle production of H_2 was maintained for over 50 h until cysteine was exhausted. Notably, *D. desulfuricans* cells in the biohybrid system were no longer viable after 24 h of light irradiation (Supporting information Figure S4), although the system continued to produce H_2 for more than 10 days. This indicates that although the cells are not able to replicate their enzymatic machinery continues to work, and thus most energy absorbed is used for H_2 production.

Conclusion

The present work reveals that *D. desulfuricans* is an excellent biological catalyst for self-photosensitization with CdS nanoparticles and generation of biohybrids with high activity for H_2 production from visible light, even in the absence of an electron shuttle. These biohybrids present excellent stability under irradiation and an apparent quantum yield that exceeds most reported systems, and thus are strong candidates for the development of new biotechnological processes for sustainable H_2 production.

Acknowledgements

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Conflict of interest

The authors declare no conflict of interest.

Keywords: biohybrid systems · hydrogen production · nanoparticles · photocatalysis · sulfate-reducing bacteria

[1] I. Staffell, D. Scamman, A. Velazquez Abad, P. Balcombe, P. E. Dodds, P. Ekins, N. Shah, K. R. Ward, *Energy Environ. Sci.* **2019**, *12*, 463 – 491.
[2] J. H. Kim, D. Hansora, P. Sharma, J. W. Jang, J. S. Lee, *Chem. Soc. Rev.* **2019**, *48*, 1908 – 1971.
[3] X. Fang, S. Kalathil, E. Reisner, *Chem. Soc. Rev.* **2020**, *49*, 4926 – 4952.
[4] S. Cestellos-Blanco, H. Zhang, J. M. Kim, Y. Shen, P. Yang, *Nat. Catal.* **2020**, *3*, 245 – 255.
[5] P. C. Sahoo, D. Pant, M. Kumar, S. K. Puri, S. S. V. Ramakumar, *Trends Biotechnol.* **2020**, *38*, 1245 – 1261.
[6] K. A. Brown, P. W. King, *Photosynth. Res.* **2020**, *143*, 193 – 203.
[7] S. H. Lee, D. S. Choi, S. K. Kuk, C. B. Park, *Angew. Chem. Int. Ed.* **2018**, *57*, 7958 – 7985; *Angew. Chem.* **2018**, *130*, 8086 – 8116.
[8] A. Bachmeier, F. Armstrong, *Curr. Opin. Chem. Biol.* **2015**, *25*, 141 – 151.
[9] E. E. Moore, V. Andrei, S. Zacarias, I. A. C. Pereira, E. Reisner, *ACS Energy Lett.* **2020**, *5*, 232 – 237.
[10] F. Zhao, P. Wang, A. Ruff, V. Hartmann, S. Zacarias, I. A. C. Pereira, M. M. Nowaczyk, M. Rögner, F. Conzuelo, W. Schuhmann, *Energy Environ. Sci.* **2019**, *12*, 3133 – 3143.
[11] P. W. King, *Biochim. Biophys. Acta Bioenerg.* **2013**, *1827*, 949 – 957.
[12] C. Tapia, S. Zacarias, I. A. C. Pereira, J. C. Conesa, M. Pita, A. L. De Lacey, *ACS Catal.* **2016**, *6*, 5691 – 5698.
[13] A. A. Krasnovsky, V. V. Nikandrov, *FEBS Lett.* **1987**, *219*, 93 – 96.
[14] Y. Honda, H. Hagiwara, S. Ida, T. Ishihara, *Angew. Chem. Int. Ed.* **2016**, *55*, 8045 – 8048; *Angew. Chem.* **2016**, *128*, 8177 – 8180.
[15] Y. Honda, M. Watanabe, H. Hagiwara, S. Ida, T. Ishihara, *Appl. Catal. B* **2017**, *210*, 400 – 406.
[16] B. Ramprakash, A. Incharoensakdi, *Int. J. Hydrogen Energy* **2020**, *45*, 6254 – 6261.
[17] S. F. Rowe, G. Le Gall, E. V. Ainsworth, J. A. Davies, C. W. J. Lockwood, L. Shi, A. Elliston, I. N. Roberts, K. W. Waldron, D. J. Richardson, T. A. Clarke, L. J. C. Jeuken, E. Reisner, J. N. Butt, *ACS Catal.* **2017**, *7*, 7558 – 7566.
[18] K. K. Sakimoto, A. B. Wong, P. Yang, *Science* **2016**, *351*, 74 – 77.
[19] N. Kornienko, J. Z. Zhang, K. K. Sakimoto, P. Yang, E. Reisner, *Nat. Nanotechnol.* **2018**, *13*, 890 – 899.
[20] N. Kornienko, K. K. Sakimoto, D. M. Herlihy, S. C. Nguyen, A. P. Alivisatos, C. B. Harris, A. Schwartzberg, P. Yang, *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 11750 – 11755.
[21] R. Zhang, Y. He, J. Yi, L. Zhang, C. Shen, S. Liu, L. Liu, B. Liu, L. Qiao, *Chem* **2020**, *6*, 234 – 249.
[22] M. Kumar, P. C. Sahoo, S. Srikanth, R. Bagai, S. K. Puri, S. S. V. V. Ramakumar, *Bioresour. Technol.* **2019**, *272*, 300 – 307.
[23] J. Ye, J. Yu, Y. Zhang, M. Chen, X. Liu, S. Zhou, Z. He, *Appl. Catal. B* **2019**, *257*, 117916.
[24] B. Wang, Z. Jiang, J. C. Yu, J. Wang, P. K. Wong, *Nanoscale* **2019**, *11*, 9296 – 9301.
[25] B. Wang, C. Zeng, K. H. Chu, D. Wu, H. Y. Yip, L. Ye, P. K. Wong, L. Ye, C. Zeng, K. H. Chu, D. Wu, H. Y. Yip, *Adv. Energy Mater.* **2017**, *7*, 1700611.
[26] Z. Jiang, B. Wang, J. C. Yu, J. Wang, T. An, H. Zhao, H. Li, S. Yuan, P. K. Wong, *Nano Energy* **2018**, *46*, 234 – 240.
[27] W. Wei, P. Sun, Z. Li, K. Song, W. Su, B. Wang, *Sci. Adv.* **2018**, *4*, 1 – 7.

[28] I. A. C. Pereira, A. R. Ramos, F. Grein, M. C. Marques, S. M. da Silva, S. S. Venceslau, *Front. Microbiol.* **2011**, *2*, 1 – 22.
[29] W. Lubitz, H. Ogata, O. Ru, E. Reijerse, O. Rüdiger, E. Reijerse, O. Ru, E. Reijerse, *Chem. Rev.* **2014**, *114*, 4081 – 4148.
[30] M. Martins, I. A. C. Pereira, *Int. J. Hydrogen Energy* **2013**, *38*, 12294 – 12301.
[31] H. Korbekandi, S. Iravani, S. Abbasi, *Crit. Rev. Biotechnol.* **2009**, *29*, 279 – 306.
[32] M. Martins, C. Mourato, S. Sanches, J. P. Noronha, M. T. B. Crespo, I. A. C. Pereira, *Water Res.* **2017**, *108*, 160 – 168.
[33] M. Martins, C. Mourato, F. O. Morais-Silva, C. Rodrigues-Pousada, G. Voordouw, J. D. Wall, I. A. C. Pereira, *Appl. Microbiol. Biotechnol.* **2016**, *100*, 8135 – 8146.
[34] C. Hamilton, S. Hiligsmann, L. Beckers, J. Masset, A. Wilmotte, P. Thonart, *Int. J. Hydrogen Energy* **2010**, *35*, 1089 – 1098.
[35] B. E. Logan, R. Rossi, A. Ragab, P. E. Saikaly, *Nat. Rev. Microbiol.* **2019**, *17*, 307 – 319.
[36] J. Xiong, D. Chan, X. Guo, F. Chang, M. Chen, Q. Wang, X. Song, C. Wu, *Appl. Microbiol. Biotechnol.* **2020**, *104*, 5579 – 5591.
[37] L. Cheng, Q. Xiang, Y. Liao, H. Zhang, *Energy Environ. Sci.* **2018**, *11*, 1362 – 1391.
[38] S. Cao, L. Piao, X. Chen, *Trends Chem.* **2019**, 1 – 14.
[39] M. Raouf Hosseini, M. Nasiri Sarvi, *Mater. Sci. Semicond. Process.* **2015**, *40*, 293 – 301.
[40] G. Dong, H. Wang, Z. Yan, J. Zhang, X. Ji, M. Lin, R. A. Dahlgren, X. Shang, M. Zhang, Z. Chen, *Sci. Total Environ.* **2020**, *740*, 140080.
[41] P. Chellamuthu, K. Naughton, S. Pirbadian, K. P. T. Silva, M. S. Chavez, M. Y. El-naggar, J. Boedicker, *Front. Microbiol.* **2019**, *10*, 00938.
[42] X. Xiao, X. B. Ma, H. Yuan, P. C. Liu, Y. Bin Lei, H. Xu, D. L. Du, J. F. Sun, Y. J. Feng, *J. Hazard. Mater.* **2015**, *288*, 134 – 139.
[43] T. Trindade, P. O'Brien, N. L. Pickett, *Chem. Mater.* **2001**, *13*, 3843 – 3858.
[44] R. Vogel, P. Hoyer, H. Weller, *J. Phys. Chem.* **1994**, *98*, 3183 – 3188.
[45] N. J. Kotloski, J. A. Gralnick, *MBio* **2013**, https://doi.org/10.1128/mBio.00553-12.
[46] L. C. Seefeldt, D. J. Arp, *J. Bacteriol.* **1989**, *171*, 3298 – 3303.
[47] H. Zhang, H. Liu, Z. Tian, D. Lu, Y. Yu, S. Cestellos-blanco, K. K. Sakimoto, P. Yang, *Nat. Nanotechnol.* **2018**, *13*, 900 – 905.

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