Carbon nitride–TiO$_2$ hybrid modified with hydrogenase for visible light driven hydrogen production†

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A system consisting of a [NiFeSe]$-$hydrogenase (H$_2$ase) grafted on the surface of a TiO$_2$ nanoparticle modified with polyheptazine carbon nitride polymer, melan (CN$_x$) is reported. This semi-biological assembly shows a turnover number (TON) of more than 5.8 $ \times $ 10$^5$ mol H$_2$ (mol H$_2$ase)$^{-1}$ after 72 h in a sacrificial electron donor solution at pH 6 during solar AM 1.5 G irradiation. An external quantum efficiency up to 4.8% for photon-to-hydrogen conversion was achieved under irradiation with monochromatic light. The CN$_x$–TiO$_2$–H$_2$ase construct was also active under UV-free solar light irradiation ($\lambda > 420$ nm), where it showed a substantially higher activity than TiO$_2$–H$_2$ase and CN$_x$–H$_2$ase due, in part, to the formation of a CN$_x$–TiO$_2$ charge transfer complex and highly productive electron transfer to the H$_2$ase. The CN$_x$–TiO$_2$–H$_2$ase system sets a new benchmark for photocatalytic H$_2$ production with a H$_2$ase immobilised on a noble- and toxic-metal free light absorber in terms of visible light utilisation and stability.

Polymeric carbon nitride (polyheptazine or melon, herein CN$_x$) is a promising visible-light absorber for the photocatalytic generation of H$_2$. We have recently reported the use of CN$_x$ as a light harvesting material in combination with a H$_2$ase and a H$_2$ase-inspired synthetic Ni catalyst for solar H$_2$ generation. The CN$_x$–H$_2$ase system showed sustained catalysis with a turnover number (TON) of more than 50 000 after 72 h solar light irradiation. However, this hybrid system suffered from a weak interaction between the H$_2$ase and the CN$_x$ surface, and consequently, poor electron transfer from CN$_x$ to the H$_2$ase. Furthermore, CN$_x$–H$_2$ase only showed efficient H$_2$ production up to wavelengths of approximately 420 nm and therefore only limited visible light harvesting capabilities.

Here, we selected a hybrid material consisting of TiO$_2$ (Hombikat UV 100, anatase, BET surface area: 300 m$^2$ g$^{-1}$, crystallite size < 10 nm) surface-modified with CN$_x$ polymer as a light absorbing hybrid material for the photocatalytic system with a H$_2$ase for three main reasons (Fig. 1; see ESI and Fig. S1† for synthesis and characterisation). Firstly, CN$_x$–TiO$_2$ can be readily prepared on a gram scale by heating TiO$_2$ nanoparticles with urea, an inexpensive and sustainable material.

Secondly, CN$_x$–TiO$_2$ provides us with substantially improved solar light harvesting performance compared to individual CN$_x$ and TiO$_2$. Band gap excitation of TiO$_2$ (pathway 1; Fig. 1) efficiently utilises the UV spectrum (band gap of 3.2 eV for anatase TiO$_2$ with CB$_{TiO_2}$ at approximately $-0.6$ V vs. NHE at pH 6).$^{13}$ A significant portion of the visible spectrum is utilised with CN$_x$–TiO$_2$ as it can, upon photo-excitation of CN$_x$, perform photoinduced electron transfer from the LUMO$_{CN_x}$ to CB$_{TiO_2}$ (pathway 2).
In addition, direct optical electron transfer can occur from the HOMO$_{CNx}$ (with contributions of molecular orbitals formed upon interaction of CN$_x$ with TiO$_2$) directly to the CB$_{TiO2}$ (pathway 3), extending the absorption even further into the visible region (up to 540 nm). This absorption pathway 3 is based on strong coupling between CN$_x$ covalently grafted onto TiO$_2$, resulting in strong charge-transfer absorption. Conclusive evidence of this charge-transfer includes previously reported spectroscopic, photoelectrochemical, and theoretical investigations.  

Thirdly, the H$_2$ evolution catalyst employed in this study, Desulfomicrobium baculatum (Dmb) [NiFeSe]–hydrogenase is not only known for its high H$_2$ evolution activity, lack of H$_2$ inhibition and O$_2$-tolerance, but also for its titaniaphilicity. This high affinity of the enzyme to adsorb strongly to TiO$_2$ stems presumably from a protein surface rich in glutamatic and aspartic acid residues close to the distal [Fe$_4$S$_4$] cluster, which act as anchor sites to TiO$_2$ and allow for stable binding and efficient electron flow into the hydrogenase active site (Fig. 1A). Thus, the CN$_x$–TiO$_2$ hybrid is expected to support a more robust H$_2$ase-particle interaction than with CN$_x$ alone, which would result in improved charge transfer and ultimately increased catalytic turnover for H$_2$ production.

### Results and Discussion

Photocatalytic systems were assembled by dispersing CN$_x$–TiO$_2$ particles in an aqueous electron donor solution (0.1 M; 2.98 mL) in a photoreactor vessel (headspace volume: 4.74 mL; see ESI† for experimental details). The vessel was sonicated under air (15 min) before sealing and purging with an inert gas (2% CH$_4$ in N$_2$). The H$_2$ase (16.5 μL, 3 μM) was then added and the photoreactor purged again to ensure anaerobic conditions. The stirred suspension was irradiated at 25 °C with a solar light simulator (air mass 1.5 global filter, I = 100 mW cm$^{-2}$) and the headspace H$_2$ was quantified at regular time intervals by gas chromatography against the internal CH$_4$ standard. The conditions were optimised for maximum turnover frequency (TOF$_{H2ase}$) by varying the electron donor and pH of the solution (Table S1; Fig. S2 and S3†). Optimised conditions consisted of ethylenediamine tetraacetic acid (EDTA; 0.1 M) as the electron donor at pH 6. A ratio of semiconductor (5 mg unless otherwise noted) to H$_2$ase (50 pmol) was used for ease of comparison to previously reported photosystems with Dmb [NiFeSe]–H$_2$ase.  

Solar (UV-visible) irradiation ($\lambda > 300$ nm) of CN$_x$–TiO$_2$–H$_2$ase under standard conditions generated an initial TOF$_{H2ase}$ of (2.8 ± 0.3) $\times$ 10$^{-5}$ a$^{-1}$ or 8 s$^{-1}$ with the production of 5.85 ± 0.59 μmol H$_2$ after 4 h and 28 ± 3 μmol H$_2$ with an overall TON$_{H2ase}$ > (5.8 ± 0.6) $\times$ 10$^2$ after 72 h (Fig. 2 and S4†). Negligible amounts of H$_2$ were detected in the absence of H$_2$ase, CN$_x$–TiO$_2$ or EDTA. UV band gap excitation of TiO$_2$ did not result in the accumulation of O$_2$, which suggests that holes generated upon UV band gap excitation of TiO$_2$ are either efficiently quenched by EDTA directly or scavenged after being trapped by CN$_x$.

To qualitatively determine the contributions from the three excitation pathways in Fig. 1B, irradiation was also performed with different long-pass filters. The CN$_x$–TiO$_2$–H$_2$ase system was studied under visible light irradiation at $\lambda > 420$ nm to study the contribution of CN$_x$ to light absorption (pathways 2 & 3) without Fig. 2. Photocatalytic H$_2$ production with Dmb [NiFeSe]–H$_2$ase (50 pmol) with CN$_x$–TiO$_2$ (5 mg) in EDTA (pH 6, 0.1 M, 3 mL) under AM 1.5G irradiation at an intensity of 1 Sun at $\lambda > 300$, 420 and 455 nm.

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**Fig. 1** (A) Schematic representation of photo-H$_2$ production with Dmb [NiFeSe]–H$_2$ase (PDB ID : 1CC1; $^a$) on CN$_x$–TiO$_2$ suspended in water containing EDTA as a hole scavenger. (B) Irradiation of CN$_x$–TiO$_2$ can result in photo-induced electron transfer by three distinct pathways: (1) TiO$_2$ band gap excitation (2) excitation of CN$_x$ (HOMOCN$_x$ – LUMOCN$_x$), followed by electron transfer from LUMOCN$_x$ into the conduction band of TiO$_2$ (CB$_{TiO2}$). (3) Charge transfer excitation with direct optical electron transfer from HOMOCN$_x$ to CB$_{TiO2}$. The CB$_{TiO2}$ electrons generated through pathways 1 to 3 are then transferred via the [Fe$_4$S$_4$] clusters to the [NiFeSe] H$_2$ase active site.

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**Fig. 2** Photocatalytic H$_2$ production with Dmb [NiFeSe]–H$_2$ase (50 pmol) with CN$_x$–TiO$_2$ (5 mg) in EDTA (pH 6, 0.1 M, 3 mL) under AM 1.5G irradiation at an intensity of 1 Sun at $\lambda > 300$, 420 and 455 nm.
the contribution of intrinsic absorption by TiO₂ (pathway 1). A photoactivity with an initial TOFₜₐₙₐₜ of 6353 ± 633 h⁻¹ was observed, which results in the generation of 1.31 ± 0.13 μmol H₂ after 4 h. After 72 h, 13 ± 1 μmol of H₂ were generated with a TONₜₐₙₐₜ of more than (2.6 ± 0.3) × 10² (Fig. 2).

Subsequently, irradiation was carried out at λ > 455 nm to investigate the contribution of the direct charge-transfer from the HOMO-CN to CB₉ZrO₂ to the photoactivity. A TOFₜₐₙₐₜ of 1096 ± 175 h⁻¹ with the evolution of 0.26 ± 0.06 μmol H₂ after 4 h and 2.9 ± 1.6 μmol H₂ after 72 h was observed, which corresponds to 17% of the visible light activity. This suggests that all three pathways in Fig. 1B contribute to the UV-vis band gap excitation of TiO₂ dominates the absorption of the hybrid material.

Visible-light response of CN–TiO₂ photoactivity was approximately 17% of the visible light activity. This suggests that all three pathways in Fig. 1B contribute to the UV-vis band gap excitation of TiO₂ (pathway 1). A TONₜₐₙₐₜ of 14852 mol H₂ a with UV-visible light (> 300 nm, Table S1). Under visible light irradiation (λ > 300 nm, Table S1†). Under visible light irradiation (λ > 420 nm), a TONₜₐₙₐₜ of 2375 ± 267 was observed after 4 h and no H₂ was produced at λ > 455 nm, demonstrating the substantially enhanced activity with CN₉–TiO₂–H₂ase compared to CN₉–H₂ase at all wavelengths (Fig. S4†).

Experiments were also performed with TiO₂–H₂ase. While the system showed comparable activity under UV-visible irradiation due to efficient band gap excitation of TiO₂ (pathway 1), it showed significantly reduced activity under visible only irradiation at λ > 420 nm and displayed negligible H₂ yields at λ > 455 nm compared to CN₉–TiO₂–H₂ase (Fig. S4†). Thus, UV-band gap excitation of TiO₂ dominates the absorption of the CN₉–TiO₂–H₂ase hybrid material under UV-light irradiation, which becomes less significant under visible irradiation.

The effect of light intensity on the photocatalytic activity (λ > 300 nm) was studied by employing neutral density filters. A photoactivity of approximately 90% remained when employing a 50% absorbance filter (50 mW cm⁻²) and 44% of activity remained with an 80% filter (20 mW cm⁻²; Fig. S5†). The initial non-linear decrease in activity implies that the system is not limited by light at 1 Sun intensity as has been observed previously with synthetic H₂ evolution catalyst-modified Ru dye-sensitised TiO₂ systems.¹⁶

The CN₉–TiO₂–H₂ase system sets a new benchmark for visible light driven and prolonged H₂ production with a heterogenised H₂ase without the need for expensive or toxic materials.¹⁴,⁹ A part of this improvement can be attributed to the direct optical electron transfer (pathway 3) within CN₉–TiO₂, which draws the absorption of solar light significantly into the visible spectrum.

The enzyme loading onto CN₉–TiO₂ was calculated based on the BET surface area of 111 m² g⁻¹, a crystallite surface area of ~314 nm² per particle and an estimation that approximately one-quarter of the surface area of TiO₂ is accessible for the enzyme to adsorb. This equates to ~0.1 H₂ase per particle of CN₉–TiO₂. The approximate 1 : 10 enzyme : particle ratio allows the H₂ase to function at the maximum rate (i.e., TOF) as the maximum electron flux of conduction band electrons is directed towards a single enzyme. To qualitatively determine the amounts of surface-bound and solubilised H₂ase in the optimised system, H₂ase (50 pmol) was loaded onto CN₉–TiO₂ (5 mg) in aqueous EDTA solution by stirring under N₂ for 15 min. The suspension was centrifuged and the supernatant decanted (see ESI† for experimental details). The CN₉–TiO₂–H₂ase pellet was re-dispersed in fresh EDTA solution (3 mL, 0.1 M, pH 6) and the photocatalytic vessel purged with 2% CH₄ in N₂. The suspension was then irradiated (λ > 420 nm) and H₂ production monitored (Fig. 3). The H₂ production activity was nearly identical to a sample that was not centrifuged, both in the presence and absence of methyl viologen (MV²⁺, see below), indicating that attachment of H₂ase to CN₉–TiO₂ is essentially quantitative. The substantially improved adsorption of the enzyme on the TiO₂ surface compared to the inert CN₉ polymer therefore also contributes to the increased activity of CN₉–TiO₂–H₂ase compared to CN₉–H₂ase. Previously an 88% decrease in photoactivity was observed with the poorly interacting CN₉–
H₂ase after centrifugation and re-dispersion in fresh electron donor buffer.²

The external quantum efficiency (EQE) of the CN₅-TiO₂-H₂ase system was measured by applying narrow band pass filters (λ = 360 ± 10 nm; I = 2.49 mW cm⁻² and 400 ± 10 nm; I = 4.34 mW cm⁻²; see ESIF for experimental details). UV-irradiation gave an EQE of approximately 4.8% and under visible irradiation an EQE of 6.51% was obtained. These values are more than a 10-fold improvement over the UV and visible EQE for the CN₅-H₂ase system,²⁷ which can be attributed to the improved light absorption (Fig. S6†) and increased electron transfer rate due to adsorption of the H₂ase onto the particle surface.

We previously showed that a significantly increased photoactivity was observed under standard conditions using CN₅-H₂ase upon addition of an excess of the redox mediator MV²⁺, producing up to 77 μmol H₂ after 69 h of UV-visible irradiation.³ A long-term experiment with H₂ase (50 pmol), CN₅-TiO₂ (5 mg) and added MV²⁺ (5 μmol) in aqueous EDTA (0.1 M) at pH 6 was performed with both λ > 300 nm light and with visible light only (λ > 420 nm). Under UV-visible irradiation after 72 h, the CN₅-TiO₂-MV-H₂ase system produced 193 μmol H₂ with a TOFₜₙₕₑₜₜ of > 3.8 × 10⁸ and an initial TOFₜₙₜₜ of 35 s⁻¹ (Fig. S7†). Under visible-light only, 66 μmol H₂ was produced with a TOFₜₙₜₜ of 1.3 × 10⁶ and an initial TOFₜₙₜₜ of 9 s⁻¹ (Fig. S8†). The ratio of the amount of hydrogen produced in the presence and absence of MV²⁺ can be used to estimate the relative efficiency of the charge transfer from material to H₂ase. Under full spectrum irradiation (λ > 300 nm) with CN₅-H₂ase the ratio was found to be 22, whereas for both TiO₂-H₂ase and CN₅-TiO₂-H₂ase systems the ratio was 5. This strongly supports the fact that there is a significant improvement in the charge transfer from a TiO₂-based material to H₂ase. In addition, this ratio remains constant when the wavelength of light used is restricted to the visible region (λ > 420 nm).

The H₂ production rates in the presence of MV²⁺ are significantly higher than those obtained in the absence of MV²⁺. The blue colour of the vials containing MV²⁺ is indicative of the formation of reduced MV⁺ in solution (Fig. S9†). By comparison, addition of MV²⁺ to the previously reported Ru-dye-sensitised TiO₂-H₂ase system caused a slight decrease in activity, which was attributed to the decreased availability of electrons for the H₂ase and the absorption of incident photons by MV⁺.²⁸ Here, solubilised MV⁺ does not limit light absorption by CN₅-TiO₂ significantly and is able to efficiently donate electrons to surface-bound H₂ase, resulting in increased H₂ production. This result implies that interfacial electron transfer from CN₅-TiO₂ to H₂ase is still not fully optimised in this system, where the orientation of the H₂ase is not fully ‘directed’. Ideally, the distance from the CN₅-TiO₂ surface to the [Fe₅S₄] electron transport chain should be minimised and an improved orientation of the enzyme would allow trapping of CB₄-TiO₂ electrons more efficiently for maximised turnover.²⁹

Favourable electron transfer kinetics at the CN₅-TiO₂-H₂ase interface can be assumed based on previous reports. Electron transfer in the order of 10⁷ s⁻¹ was reported from CdS nanorods to an [FeFe]-H₂ase isolated from Clostridium acetobutylicum.²⁶ In addition, a long lived photo-excited state lifetime of τ₂/₉ ~ 0.8 s was previously reported for TiO₂ conduction band electrons in a photocatalytic system with Ru dye-sensitised TiO₂ and electron transfer to co-immobilised molecular cobaloxime catalysts occurred with τ₂/₉ ~ 5 to 50 μs.²⁸ Based on these reports, we can assume that a reasonably long-lived TiO₂ conduction band electron is generated and that H₂ase is capable of readily collecting these electrons.

Conclusions

In summary, solar light driven H₂ production with a semi-biological system consisting of TiO₂ modified with polymeric CN₅ and immobilised H₂ase has been demonstrated. We have shown that by improving the surface interaction of the enzyme with the light harvesting CN₅ material, specifically by adsorption of the enzyme onto the TiO₂ surface, H₂ generation is drastically improved. Another important factor is the improved visible light absorption by direct CN₅ excitation (pathway 2) and CN₅-TiO₂ charge transfer (pathway 3), which enables high photoactivity. The CN₅-TiO₂-H₂ase assembly achieved a TOF of 8 s⁻¹ and TON of > 5.8 × 10⁵ after 72 h in the absence of an external soluble redox mediator, thereby setting a new benchmark for photochemical architectures based on abundant and non-toxic materials and a heterogenised H₂ase. The additional use of the redox mediator MV²⁺ allowed for the photo-generation of H₂ with a TOF of 35 s⁻¹ and a TON of > 3.8 × 10⁶. This work advances the use of hybrid photocatalytic schemes by integrating highly active electrocatalysts with advanced light absorbing materials such as CN₅-TiO₂, which is shown to be compatible with H₂ases in aqueous solution.

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Notes and references


