Photocatalytic Hydrogen Production using Polymeric Carbon Nitride with a Hydrogenase and a Bioinspired Synthetic Ni Catalyst**

Christine A. Caputo, Manuela A. Gross, Vincent W. Lau, Christine Cavazza, Bettina V.Lotsch, and Erwin Reisner*

Abstract: Solar-light-driven H₂ production in water with a [NiFeSe]-hydrogenase (H₂ase) and a bioinspired synthetic nickel catalyst (NiP) in combination with a heptazine carbon nitride polymer, melon (CNₓ), is reported. The semibiological and purely synthetic systems show catalytic activity assisted by a metallic, synthetic, or enzymatic electrocatalyst. These systems typically contain expensive, inefficient, and/or unstable components, but high performance solar fuel devices need to be constructed from parts without these limitations.

Efficient and noble metal-free water photolysis using sunlight is a primary focus of research to advance sustainable solar energy generation. Photocatalytic H₂ production can be achieved by employing hybrid systems with a solid-state light absorber such as an inorganic semiconductor assisted by a metallic, synthetic, or enzymatic electrocatalyst. These systems typically contain expensive, inefficient, and/or unstable components, but high performance solar fuel devices need to be constructed from parts without these limitations.

Hydrogenases (H₂ases) are H₂-cycling enzymes and are by far the most efficient noble-metal-free electrocatalysts for H₂ generation with an unrivalled turnover frequency (TOF) benchmark of more than 10⁶ s⁻¹ even at a modest overpotential. This excellent electrocatalytic activity of H₂ases was exploited in photocatalytic schemes with a light absorber in the absence of a soluble redox mediator: a homogeneous photocatalytic system with a molecular organic dye and semiheterogeneous systems, in which the H₂ase is immobilized on Ru dye-sensitized TiO₂ nanoparticles, and on Cd-containing quantum dots, displaying excellent photocatalytic activity in sacrificial schemes.

An efficient class of H₂ase-inspired synthetic catalysts containing non-noble metal centers have been developed by DuBois and co-workers. They possess a Ni bis(diphosphine) ligand core bearing pendant amino groups, which, much like those found in the active site of [FeFe]-H₂ases, can act as catalytically active proton relays in the second coordination sphere of the 3d metal center. Photocatalytic H₂ generation with such Ni bis(diphosphine) catalysts has only been achieved in combination with a costly Ru dye in purely aqueous solution.

Amorphous polymeric carbon nitride (CNₓ) with a poly-tri-s-triazine (polyheptazine) building block (often referred to as melon or g-C₃N₄) has recently emerged as an attractive light absorber such as an inorganic semiconductor assisted by a metallic, synthetic, or enzymatic electrocatalyst. It can be easily synthesized by condensation of cyanamide, dicyandiamide, or melamine at elevated temperatures and displays high activities and photostability of more than 72 h. The material has well-suited band positions for water splitting and a band gap of approximately 2.7 eV with a conduction band potential at −0.8 V vs. RHE. Co-catalyst integration of non-noble metals, Pt, Ni(TEOA)₂⁺ (TEOA = triethanolamine), and cobaloximes with CNₓ has previously been used as a strategy to enhance H₂ evolution rates.

In this study, we report a photocatalytic CNₓ−enzyme hybrid system for visible-light-driven H₂ generation (Figure 1). This CNₓ−H₂ase hybrid assembly operates in an
H2ase suspension was photoactive for 48 h, whereupon the TOF of H2ase was 5532 mol H2ase per mol H2 (Table S1). Irradiation of CNx resulted in a linear increase in overall H2 generation with an unchanged TOFh2ase (Table S1, Figure S6). Decreasing the solar light intensity with neutral density filters from 100 to 20 mW cm⁻² did not result in a significant reduction of the photoactivity, although a further reduction to 20 mW cm⁻² resulted in approximately 40% decreased activity (Table S2; Figure S7). These experiments suggest that the optimized CNx–H2ase system is not limited by light absorption at CNx, and that support that enzyme adsorption and interaction with the CNx is performance limiting (see below).

The CNx–H2ase system was also studied under visible light irradiation (λ > 420 nm). A decrease in photoactivity was observed giving rise to a TOFh2ase of (768 ± 77) h⁻¹, which corresponds to 14% of the activity under UV/Vis irradiation (Figure 2). This can be attributed to the significantly reduced light absorption of CNx above 420 nm (Figure S8). The external quantum efficiency (EQE) of the system was determined by irradiation of samples under standard conditions using a monochromatic LED light source at two wavelengths (λ = 365 nm, I = 3.5 mW cm⁻² and λ = 460 nm, I = 47 mW cm⁻²). UV-irradiation gave an unoptimized EQE of approximately 5 × 10⁻² %, whereas an EQE of 5 × 10⁻³ % was obtained at λ = 465 nm (Figure S8).
A centrifugation test was performed to gain insight into the strength of interaction between the enzyme and CNx particles. First, H2 production was monitored for 2 h with CNx–H2ase under standard conditions. The suspension was then centrifuged (5000 rpm, 5 min) followed by washing the pellet with water and redispersion of the particles in aqueous EDTA (0.1 M, pH 6). This suspension was then irradiated again after purging the headspace with 2% CH4 in N2. The remaining activity of this mixture was 12% relative to the activity prior to centrifugation, indicating that a relatively weak interaction suffices for electron transfer to occur from CNx to the H2ase. Physical adsorption of the H2ase on the CNx surface can be expected and we speculate that the H2ase[16] may form hydrogen bonds with the –NH–, terminal –NH2 or Lewis basic heptazine edge nitrogens in CNx.[10a,17] The isoelectric point of CNx was determined by zeta potential measurements as 3.3[18] and, at pH 6, the surface of CNx is therefore negatively charged (∼−15 mV) (Figure S4).

Although the direct electron transfer was observed from the photoexcited CNx to the H2ase, the CNx–H2ase system displayed a significantly increased photoactivity under standard conditions upon addition of an excess of the redox mediator, methyl viologen (MV).[19] producing up to 18.7 μmol H2 after 4 h (Figure S9). A long-term experiment with H2ase (50 pmol), CNx (5 mg), and added MV (5 μmol) in aqueous EDTA (0.1 M) at pH 6 was also performed. The photoreactor was purged with 2% CH4/N2 after 24 and 48 h and additional MV (5 μmol) was added at the same time intervals. After 69 h, the CNx–MV–H2ase system produced 77 μmol H2 with a TON of 1.5 × 10^6 and an initial TOF of 12.3 s⁻¹ (Figure S10). Replenishment of MV was required due to decomposition of the organic mediator during irradiation. The substantially increased H2 production activity in the presence of MV suggests that the electron transfer from CNx to H2ase is not yet fully optimized, presumably due to weak and nonspecific interactions at the CNx–H2ase interface.

Steady-state photoluminescence (PL) measurements were also performed with the CNx in suspension upon photoexcitation at λ = 365 nm and following the PL emission at 450 nm (Figure S11). The PL emission of sonicated CNx (0.22 g·L⁻¹ in 0.1 M EDTA pH 6) is more strongly quenched upon addition of 50 pmol MV compared to 50 pmol H2ase. These results further support that the photoinduced electron transfer from CNx to MV is more efficient than that to the H2ase.

The reported semibiological hybrid system provides a novel “per active site” activity benchmark for a cocatalyst on a CNx material.[5g,11a,b,20] Photocatalytic H2 generation schemes previously reported with H2ases and other light absorbers show a high TOF (H2ase) (approximately 10^4 h⁻¹), but these systems rely on an expensive (Ru dye), toxic (Cd-based quantum dot), and/or fragile (organic dye) visible light absorber.[5a,b,6c] This study demonstrates that the biocompatibility of CNx can be exploited to overcome these limitations and that by improving the coupling of CNx to the H2ase, the photoactivity will be further enhanced.

Successful H2 production with CNx–H2ase prompted us to investigate a water-soluble and functional synthetic H2ase-mimic, [Ni2(P3,P4)(PPhCH2P(O)(OH)3)2]2Br2 (NiP; Figure 1),[9] for comparison. Ni bis(diporphosine)[7a–c] complexes are among the most active H2 generation electrocatalysts and, importantly, NiP has recently been shown to act as an excellent electrocatalyst in aqueous solution.[9] The purely synthetic CNx–NiP assembly is photoactive and conditions were optimized for the highest TOFNP. Aqueous EDTA solutions (0.1 M) at pH 4.5 containing NiP (20 nmol) and suspended CNx (5 mg) were studied under simulated solar irradiation at λ > 300 nm (Table S3, Figures S12–S14).

Under these conditions, solar H2 generation by CNx–NiP gave an initial activity of (437.1 ± 43.7) μmol H2 (g CNx)⁻¹ h⁻¹ producing (2.2 ± 0.2) μmol H2 in the first hour and giving a TOFNP of (109.3 ± 10.9) mol H2 (mol NiP)⁻¹ h⁻¹. CNx–NiP was photoactive for three hours, whereupon (3.3 ± 0.4) μmol of H2 with a TON of (166.1 ± 20.6) mol H2 (mol NiP)⁻¹ was produced (Figure 3). A 64% decrease in photocatalytic H2 generation yield was observed for CNx–NiP when irradiating with λ > 420 nm instead of >300 nm solar light. Decomposition of NiP is the likely reason for the ceased activity after three hours, because the photoactivity is fully regenerated if additional NiP is added (Figure S15).

Photo-H2 generation with CNx–NiP is thus significantly higher than for previously reported CNx systems with immobilized noble-metal-free cocatalysts in aqueous solution. A TOF of <0.5 h⁻¹ and a TON of 4 was reported for a cobaltoxime, [CoCl(dimethylglyoximato)(pyridine)], after 8 h irradiation with CNx in aqueous TEOA at pH 10.4.[14b] Other systems comprising a cobaltoxime with a pyrene-functionalized pyridine[14a] and NiCl2 with TEOA and CNx[13] showed TONS of 160 and 281 and TOFs of approximately 40 and 6.7 h⁻¹, respectively, but required excess organic solvent. Previously, photo-H2 generation with NiP was only reported with a molecular Ru dye, in which a TOFNP of up to 460 h⁻¹ and a TONNP of up to 723 in pH 4.5 ascorbic acid solution were reported.[9]

The photo-H2 generation activity of CNx–NiP is dependent on the NiP concentration (Figures S13 and S14) and...
reduction of the light intensity (I) with neutral density filters has a substantial impact on the photoactivity. The NiP-based TOF decreases from (71.1 ± 7.1) h⁻¹ (I = 100%) to (32.4 ± 3.2) (I = 50%) and (13.1 ± 1.4) h⁻¹ (I = 20%; Table S4; Figure S16). The purely synthetic system is therefore limited both by catalyst concentration and light absorption. The unoptimized EQE for the CNx–NiP system was determined to be (0.37 ± 0.02)% under UV light (λ = 365 nm) and (0.04 ± 0.01)% under blue light irradiation (λ = 460 nm) after 2 h. The wavelength-dependent EQE is consistent with the decrease in light absorption by CNx at higher wavelengths (Figure S17).

Centrifugation experiments in analogy to the enzyme system were performed to examine the strength of the interaction between CNx and NiP. After centrifugation, washing, and redispersion in fresh EDTA solution, 8% of the photoactivity remained for the synthetic system implying a weak interaction between the CNx and NiP (Figure S18). Electronic absorption spectrophotometry was used to quantify the amount of NiP adsorbed to CNx. By comparing UV-visible spectra of NiP (6.7 μM; λmax = 320 and 450 nm) in aqueous EDTA solution (3 mL; 0.1 M, pH 4.5) before and after the addition of CNx and centrifugation, an estimate of approximately 20% NiP was adsorbed on CNx (Figure S19). The physical adsorption and H-bonding between the phosphonic acid groups in NiP and the terminal –NH₂ and –NH₃ groups in CNx are possible modes of interaction.[10b,17]

The addition of MV (20 μmol) to a standard photocatalytic experiment showed an approximately 20% decreased H₂ production activity. The reaction mixture turned dark blue upon irradiation, indicative of the presence of reduced MV, and implies that MV successfully scavenged electrons from the photoexcited CNx, but was unable to transfer them to NiP (Figure S20).

The comparison of the CNx–H₂ase with the CNx–NiP hybrid system shows the expected higher “per active site” activity of the enzymatic system, whereas the purely synthetic system shows an overall higher H₂ production rate due to the larger amount of NiP (20 nmol) used compared to H₂ase (50 pmol). Thus, we also studied the CNx catalyst systems with the same amount of NiP and H₂ase (200 pmol) on CNx, (5 mg) in aqueous EDTA solution (pH 4.5 and pH 7.0, respectively). At the same concentration, the enzyme (TOF = 2528 h⁻¹) greatly outperforms the NiP cocatalyst (TOF = 64 h⁻¹), demonstrating that substantial improvements are still required to develop synthetic catalysts with activities comparable to enzymes (Figure S21, Tables S1 and S3).

Finally, we photodeposited 1 wt% Pt onto CNx (5 mg) for a direct comparison of this benchmark system with CNx–H₂ase and CNx–NiP. Following a standard procedure,[12] the platinumized CNx system was irradiated with visible light (λ > 420 nm) in an aqueous 10 vol% TEOA solution, generating 94 μmol H₂(gCNx)⁻¹ h⁻¹, which corresponds to a TOFₚ of 4.3 mol H₂(mol Pt)⁻¹ h⁻¹. Thus, the CNx–H₂ase and CNx–NiP systems compare favorably when using TOF as the metrics of system performance.

In summary, solar-light-driven H₂ production with hybrid systems consisting of polymeric CNx with H₂ase and the bioinspired synthetic catalyst, NiP, has been demonstrated. The systems operate without a soluble redox mediator and are not limited by a photo-unstable or expensive dye. The semibiological CNx–H₂ase assembly achieved a record TOF of 5532 h⁻¹ and TON of >50000 after two days as a cocatalyst with CNx. The additional use of the redox mediator MV allowed for the photogeneration of H₂ with a TOF of 12.3 s⁻¹ and a TON of >1 × 10⁸, which displays the further potential of the hybrid assembly after optimization of the biomaterial interface. CNx–H₂ase also maintains respectable activity under visible light irradiation for more than 48 h. Recent investigations into improving the absorption profile of CNx in the visible range demonstrate the potential of this material and illustrate that its use as a light-harvesting material will continue to develop, as its absorption profile is further improved.[21] The entirely synthetic CNx–NiP system displays an unprecedentedly high TOF (109 h⁻¹) and TON (166) for a hybrid system made of a molecular cocatalyst with CNx in purely aqueous solution. This work advances the use of hybrid photocatalytic schemes by integrating highly active electrocatalysts with the photostable and inexpensive CNx, which is shown to be compatible with biological and bioinspired electrocatalysts, namely hydrogenases and their mimics in aqueous solution.

Keywords: carbon nitride · enzyme catalysis · hydrogen production · hydrogenases · photocatalysis

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