Accelerating NADH oxidation and hydrogen production with mid-gap states of nitrogen-rich carbon nitride photocatalyst

Highlights
Additive assisted synthesis of N-rich PCN (NPCN) with mid-gap states is proposed

About 98% of NADH oxidation is realized using NPCN photocatalyst

A photocatalytic hydrogen evolution rate of 11.18 mmol g⁻¹ h⁻¹ was achieved on NPCN

Existence of mid-gap electronic states facilitate charge carrier separation
Accelerating NADH oxidation and hydrogen production with mid-gap states of nitrogen-rich carbon nitride photocatalyst

Toshali Bhoyar, Dong Jin Kim, B. Moses Abraham, Akanksha Gupta, Nagesh Maile, Nilesh R. Manwar, Surendar Tonda, Devthade Vidyasagar, and Suresh S. Umare

SUMMARY

Regeneration of electron carriers such as NAD+/NADH is highly desirable and essential for enzymatic conversions. Here, we demonstrate a sustainable strategy for the regeneration of NAD+ as an electron carrier via photon-assisted heterogeneous catalysis. For this, a mid-gap state induced nitrogen-rich polymeric carbon nitride (NPCN) catalyst was synthesized by an additive-assisted thermal copolymerization. Utilizing NPCN as a photocatalyst presented NADH photooxidation efficiency of over 98% and a high hydrogen production rate of 11.18 mmolg⁻¹h⁻¹ with an apparent quantum yield of 9.16% (λ = 420 nm), outperforming other state-of-art metal-free photocatalysts. The experimental and theoretical simulations suggest that mid-gap states in NPCN catalyst are main platform for charge-carrier separation that enhances the overall photocatalytic performance.

INTRODUCTION

Dihydronicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide (NAD⁺) are important electron carriers in many oxidoreductase enzymatic redox conversions. Glucose, alcohol, and aldehyde dehydrogenase use coenzyme NAD⁺ as an oxidant to generate value-added gluconic acid, aliphatic aldehydes, and carbonyl compounds. However, all these conversions are accomplished at the expense of NADH/NAD⁺ coenzyme consumption, which increases the cost of the catalytic process and endows waste generation. Therefore, reducing the amount of coenzyme consumption and regeneration of NAD⁺ has been a hot topic of research interest. Despite utmost importance, regeneration of enzymatically active component NAD⁺ is scantily explored and reported. Typically, NAD⁺ regeneration is accomplished by NADH-dehydrogenase, formate dehydrogenase, or carbonyl reductase enzymes. However, these enzymes are expensive and need extreme care to maintain their stability and safety. As an alternative, non-enzymatic NAD⁺ regeneration can effectively overcome these limitations. Recently, photo/electrochemical NADH oxidation approaches were employed using a variety of electrodes. However, an over-potential of >1 V is necessary for the NADH oxidation that induces interference of other oxidizable analytes limiting their practical applications. Although the interference can be minimized by utilizing redox mediators, the process becomes complex and cost intensive. In this context, solar energy-driven photocatalysis can be a sustainable approach for NADH oxidation. Furthermore, photocatalytic NAD⁺ regeneration can be a low-cost, low-complexity, and energy-efficient technique. In the recent past, conjugated polymer hydrogel, conjugated microporous polymer nanoparticles (B-BO₃), CdS, BiVO₄, and polymeric carbon nitride (PCN) are employed for photooxidation of NADH to NAD⁺ in an aqueous medium. Nevertheless, the NADH photooxidation ability of these photocatalysts remains unsatisfactory. Therefore, it is highly desirable to develop an efficient visible light harvesting photocatalyst capable of continuously producing NAD⁺, an enzymatically active component.

Metal-free PCN-based photocatalysts with visible light harvesting ability are well known for variety of photocatalytic reactions including for CO₂ reduction, water splitting, and decontamination of pollutants. Importantly, PCN based photocatalysts are inexpensive, easy to synthesize, eco-friendly in nature, and show superior thermo-chemical stability. Although PCN type catalysts have unique physico-chemical properties, the bulk PCN photocatalyst exhibit poor photoactivity because of the rapid recombination of photoexcited charge-carriers and high surface inertness. Henceforth, reducing the recombination effect in PCN can excel the separation of electron-hole pair and enhance overall photocatalytic activity.
Defect engineering is an effective way to accelerate the dissociation of photoexcited charge carriers by modulating the electronic band structure of PCN. For instance, surface engineering of \( p \)-conjugative carbon nitride framework with additional organic molecules dramatically alters its optoelectronic properties. As a result, a new electronic configuration is imparted within the band gap known as the mid-gap/defect state. These intrinsic trap states can potentially reduce radiative charge carrier recombination and thus extend the overall visible light absorption range. The existence of a mid-gap/defect state also influences the reduction capability of PCN by means of uplifting conduction band edges. Until now, numerous efforts have been made to improve the active surface area, reduce the fast charge-carrier recombination of PCN and enhance its photocatalytic performance. Commonly used techniques include the introduction of additional nitrogen sites via the salt melt method and heterojunction construction. The presence of excess nitrogen sites also improves reduction ability, broadens light absorption, and minimizes the photo-exciton recombination; owing to the effective electron trapping by mid-gap states.\[9\]

Herein, we employed a simple additive engineering approach to induce excessive nitrogen sites and mid-gap states to the pyrolyzed PCN. A nitrogen-rich aminoguanidine hydrochloride (AGH) with a skeletal structure analogous to urea is employed as a molecular additive to synthesize nitrogen-rich PCN. This study reports a unique and facile synthesis of metal-free N-rich PCN with superior charge-carrier kinetics and large surface area. The photooxidation ability of as developed nitrogen-rich PCN (NPCN) is explored for the photo-driven NADH oxidation, hydrogen production, and degradation of model organic pollutants. The insights of catalyst design and the underlying physicochemical properties for enhanced photoactivity are discussed in detail.

**RESULTS AND DISCUSSION**

**Phase and structural analysis of catalyst**

Integration of heterogeneous photocatalysis with enzymatic catalysis has the high merit of achieving superior catalytic performance. These photo enzymatic reactions proceed through light-induced charge carrier generation by photocatalyst, charge transfer between photocatalyst and enzyme, and enzyme-assisted product conversion (Figure 1A). The charge-transfer step between photocatalyst and enzyme...
dictates the overall performance of the photoenzymatic reaction. Henceforth, optimizing charge-transfer photophysics between the photocatalyst and the enzyme is expected to increase overall reaction kinetics. We, therefore, developed an N-rich PCN catalyst to promote the rate of charge-transfer kinetics at the photocatalyst/enzyme interface. For this purpose, an N-rich PCN catalyst was synthesized by thermal pyrolysis of an N-rich precursor aminoguanidine hydrochloride (AGH). The crystal structure of bare PCN examined by the powder X-ray diffraction (XRD) pattern shows two characteristic diffraction peaks at 2θ angles of 13.14° and 27.68°, which are assigned to the (100) and (002) planes, respectively (Figure 1B). The (100) plane corresponds to intralayer repeating stacking of tri-s-triazine tectonics with an interlayer (d-spacing) of 0.659 nm; which is consistent with the d-spacing of in-planar repeating tri-s-triazines. The high intensity (002) plane corresponds to interlayer stacking of tri-s-triazine units with an interlayer spacing of 0.322 nm, which indicates the formation of a graphitic carbon nitride structure. Nonetheless, the peak intensity corresponding to the (002) plane decreases significantly and shifts to a higher 2θ value compared to PCN suggesting an amorphous and distorted polymeric network with increased interlayer spacing and smaller crystallite size (Table 1). The diffraction peaks of the defective nitrogen-rich carbon nitride (NPCN) derived from a mixture of AGH, melamine, and urea are consistent with those of PCN. Nonetheless, the peak intensity corresponding to the (002) plane decreases significantly and shifts to a higher 2θ angle of 27.82° with an interlayer spacing of 0.321 nm. The (002) peak’s low intensity implies an interplanar disordered polymeric structure with short periodicity, whereas a smaller interlayer distance indicates compact interlayer stacking. In addition, the crystallite size estimated using the Scherrer equation revealed a decrease in crystallite size with the incorporation of AGH (Table 1). This implies that adding AGH during the carbon nitride polymerization significantly influences its crystal structure.

The solid-state 13C cross-polarized magic angle spinning (CP-MAS) nuclear magnetic resonance (NMR) spectra of PCN revealed two signals at 164.95 and 156.36 ppm, originating from the NH2-C-N2 (C1) and C-(N)3 (C2) carbons in the heptazine core, respectively (Figure 1C). The 13C CP-MAS NMR spectrum APCN and NPCN catalysts shown similar signals comparable to the PCN, confirming the formation of the graphitic type carbon structure. On the other hand, both APCN and NPCN exhibit an increase in the peak intensity at 156.36 ppm. As a result, the C2/C1 intensity ratio increased from 0.5 (for PCN) to 0.8 and 0.7 for APCN and NPCN, respectively, indicating a change in the local carbon environment. Furthermore, the characteristic two carbon signals in NPCN catalyst are shifted to higher chemical shift values of 165.43 and 156.90 ppm. This higher chemical shift value reflects a carbon de-shielding caused by the reduced electron density on the heptazine ring owing to the enhanced nitrogen concentration.

The Fourier transform RAMAN (FT-RAMAN) spectrum of PCN presented typical skeletal vibrations of carbon nitride heterocycle at 1200–1800, 980, and 700 cm⁻¹ corresponding to the C-N stretching vibrations, symmetric N-breathing mode of heptazine unit, and in-plane bending vibration of the C-N=C linkage, respectively (Figure S1). The APCN and NPCN has shown identical peaks like that of PCN with more pronounced intensity. The NPCN catalyst disclosed a shoulder peak at 1208 cm⁻¹ signifying a change in the carbon nitride framework (Figure 1D). Apart from the signature peaks, all the three catalysts exhibit two distinct signature peaks of the D (1350 cm⁻¹) and G (1566 cm⁻¹) bands. Here, the D band denotes the degree of defect sites in the graphitic domain, whereas the G band corresponds to the sp² aromatic domain. The number of defects can be elucidated by the relative intensity ratio of Iᵩ/I₉. In the case of NPCN, the Iᵩ/I₉ ratio increased from 0.35 (PCN) to 0.41, which denotes the presence of excess defects in NPCN than PCN. The Fourier transform infrared (FT-IR) spectrum exhibits fundamental vibrational bands for PCN ca. 800, 1200–1600, and 3000–3500 cm⁻¹, which are assigned to the breathing mode of tri-s-triazine, stretching mode of CN heterocycle, and N-H vibrations of terminal NH/NH₂ groups, respectively (Figure 1E). In comparison to PCN, APCN and NPCN have nearly similar skeletal vibrational bands, confirming the retention of the PCN backbone.

### Table 1. The structural parameter and crystallite size of as-prepared PCN materials

| Catalyst | 2θ (degree) | d (nm) | FWHM (β) | Crystallite Size (nm) |
|----------|-------------|-------|----------|----------------------|
| PCN      | 27.68       | 0.322 | 0.565    | 15.13                |
| APCN     | 27.40       | 0.325 | 1.283    | 6.66                 |
| NPCN     | 27.82       | 0.321 | 0.665    | 12.86                |
Chemical state, optical response, and microstructural analysis

The X-ray photoelectron spectroscopic (XPS) analysis of the PCN, APCN, and NPCN catalyst surface revealed the presence of carbon, nitrogen, and oxygen on the surface of catalysts (Figure 2A). The high-resolution C1s spectrum of pristine PCN is deconvoluted into three peaks with a binding energy of 284.80, 286.21, and 288.24 eV, which are assigned to adventitious carbon, terminal sp² carbon atom bonded to sp³ hybridize nitrogen atom (C-NHₓ), and core sp² carbon atom bonded to sp² nitrogen atoms (N-C=N), respectively (Figure 2B). The N1s spectrum of PCN is fitted with three peaks at 398.71, 400.11, and 401.30 eV, corroborated to sp² nitrogen of the C-N=C linkage, core sp³ nitrogen atom N-(C)₃, and sp³ nitrogen of terminal C-NHₓ groups (Figure 2C). The XPS spectra of APCN and NPCN revealed similar C1s, and N1s spectra, indicating the formation of a graphitic carbon framework. Furthermore, the O1s spectrum of all the three PCNs is fitted with a single peak, likely from the absorbed moisture on the catalyst surface (Figure S2). Despite this, some peaks in C1s and N1s spectra are shifted to higher and others to lower binding energy. The variation in binding energy value describes a change in the bonding environment, which is most probably caused by the difference in the precursor. To evaluate the reason for shift in binding energy values, we calculated the C/N ratio for PCN, APCN, and NPCN catalysts. Remarkably, the carbon to nitrogen atomic ratio (C/N) of APCN (0.72) and NPCN (0.70) are found to be lower than that of PCN (0.77), signifying that the NPCN, and APCN structures contains more nitrogen atoms. The XPS quantification after excluding the O1s element, disclosed the presence of 43.80% carbon and 56.20% nitrogen in PCN. As a result, the empirical formula is estimated to be C₃N₃.9, which is close to the ideal C₃N₄ stoichiometry of carbon nitride. Besides, APCN consists of 41.73% carbon and 58.27% nitrogen with the empirical formula C₃N₄.2. Notably, the percentage of carbon and nitrogen reached 41.20% and 58.80% for NPCN,
respectively which represents an empirical formula of C$_3$N$_{4.3}$ for NPCN. The XPS quantification analysis suggests that adding AGH to the polymerization precursor increases the nitrogen concentration in the catalyst. This observation indicates that change in precursor has strong influence on the atomic concentration of the catalyst. As a result of increased nitrogen concentration, NPCN and APCN catalysts shown shift in the binding energy values.

The influence of precursors on the optical absorption ability of PCN catalyst is examined by recording the UV-Visible diffuse reflectance spectra (UV-Vis DRS). The digital photographs of the powder catalysts clearly demonstrate a significant change in color (Figure S3). As a result, compared to PCN, the UV-DRS spectrum of APCN demonstrated a bathochromic effect with an extended optical response to 600 nm (Figure 2D). However, NPCN exhibited a hypsochromic shift with respect to pristine PCN. The band gap energy values estimated from the transformed Kubelka-Munk (K-M) plot are calculated as 2.62, 1.59, and 2.76 eV for PCN, APCN, and NPCN, respectively (Figure S4A). The band gap value significantly increased for NPCN after incorporation of AGH. The increase in the band gap of NPCN catalyst might be attributed to quantum confinement effect. The crystallite size calculated for NPCN (12.86 nm) is smaller than PCN (15.13 nm) in accordance to the diffraction data (Table 1). The smaller crystallite size of NPCN supports increased band gap value due to increase in the grain boundary. Furthermore, the K-M plot demonstrates clear sub-states in PCN and APCN. Perhaps, the presence of humps in the absorption spectra in NPCN, in contrast to smooth absorption spectra of PCN indicates induced mid-gap states.

The transition energy ($E_t$) from the valence band to the mid-gap states of NPCN catalyst is calculated to be 2.47 eV from Kubelka-Munk plot. This observation implies a change in electronic features of NPCN because of the preorganization of precursors. The disorderness, defects, and band structure of the as-synthesized catalysts can be evaluated by employing Urbach energy. Therefore, the Urbach energy of catalysts is calculated using absorption coefficient and photon energy by applying the Urbach rule (Equation 1);

$$\ln \alpha = \ln \alpha_0 + \frac{h\nu}{E_u}$$

where $\alpha$, $\alpha_0$, $h\nu$, and $E_u$ represents absorption coefficient, constant, photon energy, and Urbach energy, respectively. The reciprocal slope of the curve between absorption coefficient versus photon energy gives $E_u$ value in eV. The estimated Urbach energy of NPCN (0.200 eV) is higher than that of PCN (0.193 eV) (Figures S4B–S4D). The increase in the Urbach energy value signifies disorderliness in the polymeric structure of NPCN catalyst. This could be because of the presence of mid-gap states in NPCN, that provides surface states to accommodate photogenerated electron thereby inhibiting rapid recombination of charge carriers. A detailed discussion on the mid-gap states of catalysts is presented in later sections.

The microstructure analysis of PCN using transmission electron microscopic (TEM) presented a two-dimensional (2D) aggregated structure with bent edges probably because of the reduced surface energy (Figure S5A). Similarly, APCN shows a highly dense bulk carbon nitride structure (Figure S5D). Moreover, compared to PCN and APCN, NPCN displays a 2D ultrathin nanolayered structure (Figure 2E), which is caused by the excess liberation of ammonia, and carbon dioxide gases during the thermal polymerization process. The high-resolution transmission electron microscopic (HRTEM) images of both PCN and APCN did not reflect lattice fringes suggesting amorphous nature (Figures S5B and S5E). The selected area electron diffraction (SAED) pattern further indicates the amorphous nature of PCN and APCN (Figures S5C and S5F). Whereas the HRTEM image of NPCN catalyst has shown lattice fringes of d-spacing equal to 0.319 nm corresponding to (002) plane of NPCN (Figure S6). To add, the SAED pattern exhibited concentric rings with a d-spacing of 3.2 Å, suggesting the polycrystalline nature of the sample (Figure 2F). The 2D ultrathin nanolayered structure of NPCN catalyst was further confirmed by atomic force microscopy (AFM) analysis. The average sheet thickness of the catalysts was determined to be ~3.8 nm for NPCN catalyst, thinner in comparison with PCN (154 nm) and APCN (122 nm) catalysts (Figure S7). The ultrathin nanosheets type structure of NPCN catalyst expose large number of surface-active sites which are beneficial for surface photocatalytic reactions.

**Evaluation of photocatalytic activity**

**Photocatalytic NADH oxidation and hydrogen evolution**

To demonstrate the concept of photocatalytic NAD$^+$ regeneration, the NADH oxidation reaction was performed under visible light irradiation and NADH/NAD$^+$ conversion was monitored using a UV-visible
The NADH shows two absorption bands in the UV-Vis absorption spectrum at λ: 340 nm and 260 nm. The absorption band at λ: 340 nm corresponds to the reduced NADH form originating from the n-π* transition, whereas, the high-intensity absorption band at λ: 260 nm corresponds to the oxidized NAD⁺ form, which is ascribed to the π-π* transition of the adenine ring. Therefore, NADH oxidation can be assessed by measuring absorbance at 340 nm. The NADH photooxidation over as-prepared PCNs on light irradiation was monitored after every 10 min of light irradiation. After 50 min of light irradiation, the PCN showed 33.5% NADH conversion efficiency, whereas APCN has shown nearly 60% photoconversion under similar reaction conditions (Figure 3A). Notably, the NPCN catalyst disclosed a much higher NADH conversion efficiency of 98%, under 50 min of light irradiation. The absorbance spectra of the sample recorded right after every 10 min of light irradiation demonstrate a decrease in the intensity of absorption peak at 340 nm, and increased intensity of peak at 260 nm signifying the production of NAD⁺ (Figure 3B). The photocatalytic NADH conversion to NAD⁺ can be realized by a clear change in the absorption spectra. Moreover, two control experiments were conducted in parallel to ensure the oxidation reaction as a synergistic effect of catalyst and photons. There were no indications of photooxidation behavior in the control reaction that was conducted in complete dark without light (Figure S8A).
conducted without using a catalyst also revealed no photooxidation reaction occurring over time (Figure S8B). The rate of photooxidation over the catalyst is calculated using a pseudo-first-order kinetic fit, and the photooxidation rate constant value for NPCN (0.053 min$^{-1}$) is found to be greater than that of PCN (0.008 min$^{-1}$) and APCN (0.019 min$^{-1}$), respectively (Figure 3C). A comprehensive comparison of NADH oxidation performance obtained herein has been compared with the previous reports and tabulated in Table S2. Though the NADH oxidation activity is lower than the published catalyst, we achieved NADH oxidation over purely carbon-based photocatalyst and under visible light irradiation which is focus of the work.

A qualitative assessment was further performed to confirm the NADH photooxidation to NAD$^+$. The sole structural difference between the reduced NADH and oxidized NAD$^+$ is the hydride at the H4 position of the nicotinamide group and conjugation in the pyridine ring (Refer to Figure S9A). Therefore, we obtained the $^1$H nuclear magnetic resonance (NMR) spectrum of the aqueous reaction mixture before (NR) and after (NO) photooxidation reaction between 9.50 and 5.50 ppm (Signals between 5.5-4.0 ppm were neglected because of the strong $^1$H NMR peaks of water) (Figure S9B). The $^1$H NMR spectrum of NR shown characteristic signals at 8.43 and 8.17 ppm corresponding to two protons of the adenosine ring (A2 and A8) (Figure S9C). On the other hand, NAD$^+$ demonstrates signals at chemical shift values 9.27, 9.10, 8.79, and 8.12 ppm assigned to the protons of the nicotinamide group of NAD$^+$ (H6, H2, H4, and H5) (Figures S9D and S9E). These observations are in good agreement with the previous reports of NADH oxidation,$^{1,35}$ and their appearance confirms the formation of NAD$^+$ over NPCN catalyst under light illumination.

The hydrogen evolution reaction (HER) was conducted by dispersing the catalysts in an aqueous solution of TEOA as sacrificial electron donor and 1wt% Pt as cocatalyst under visible light irradiation ($\lambda > 420$ nm). After one hour of light irradiation, the PCN and APCN catalyst generated 1.64 and 2.32 mmolg$^{-1}$ of hydrogen gas. A significant increase in photocatalytic hydrogen production amount of 12.65 mmolg$^{-1}$ was observed for NPCN under similar conditions (Figure 3D). On continuous irradiation for four hours, the amount of hydrogen production for PCN, APCN, and NPCN catalysts reached to 5.81, 6.97, and 40.32 mmolg$^{-1}$, respectively. The NPCN catalyst remarkably outperformed the PCN and APCN with a hydrogen production rate of 11.18 mmolg$^{-1}$h$^{-1}$ (Figure 3E). The apparent quantum yield (AQY) of NPCN catalyst was further calculated (refer STAR Methods for details about AQY calculation) to be 9.16% at a fixed wavelength of 420 nm, which is highly competitive with the several reported catalysts (Figure S10 and Table S3). The higher hydrogen production rate of NPCN is likely because of the excess nitrogen content and possible mid-gap states. Furthermore, the photocatalytic stability of NPCN catalyst conducted for five repetitive cycles presented a negligible decrease in the hydrogen yield after five cycles (still higher than PCN, and NPCN (Figure 3F)). This slight reduction in the H$_2$ production is probably because of the loss of surface-active sites on NPCN catalyst. Besides, the XRD and FT-IR patterns of NPCN before and after the stability test indicate that the cyclic run of 20 h does not alter the phase and chemical structure of the catalyst (Figures S11A and S11B). To add, the scanning electron microscopic (SEM) image of the recycled NPCN after 5th run exhibited no obvious change indicating excellent microstructural stability (Figures S11C and S11D). All these findings indicate that NPCN catalyst has outstanding photoactivity, structural stability, and charge carrier transport characteristics.

**Photocatalytic degradation of persistent organic pollutants**

We also studied the photooxidation performance of the catalysts for the degradation of two model persistent organic pollutants (POPs), ciprofloxacin (CIP) an antibiotic, and rhodamine B (RhB) dye. The CIP degradation performance in an aqueous solution of PCN catalyst resulted in 25% degradation over 100 min of light irradiation. Of interest, the degradation efficiency reached to 74% using NPCN catalyst (Figures 3G and S12A). The degradation rate of CIP estimated using pseudo-second-order kinetics for NPCN is found to be 0.013 min$^{-1}$, which is around 4-fold higher than that of pristine PCN (0.003 min$^{-1}$) (Figures 3H and S12B). The degradation of a model-colored pollutant RhB also suggested enhanced degradation performance for NPCN catalyst (Figures S11 and S13A). The photocatalytic control experiments performed in dark and without catalyst further confirmed degradation as the light-driven process on the catalyst’s surface. The RhB degradation rate constant of NPCN (0.085 min$^{-1}$) is further calculated and shows a higher degradation rate constant than PCN (0.003 min$^{-1}$) (Figure S13B). To check the structural stability of the as-prepared catalyst after degradation performance, the RhB degradation was carried out for four consecutive cycles. The NPCN catalyst showed excellent degradation performance for four cycles (Figure S14A).
The XRD pattern of the catalyst NPCN after the fourth cycle was found to be consistent with freshly used catalyst, indicating excellent photostability of the catalyst (Figure S14B).

**Charge carrier recombination dynamics of photocatalysts**

The charge transfer dynamics of the as-prepared PCN catalysts were analysed by the room-temperature photoluminescence (PL) spectroscopy. Notably, the as-prepared PCNs have shown a broad emission peak that indicates the origin of the multi-phonon process in which excited electrons relax via multiple paths involving several energy states within the bandgap (Figure 4A). On comparing the emission spectra it is clear that the emission intensity increases for NPCN compared to PCN. To investigate the reason for increased emission intensity, the emission spectra are fitted using a Gaussian function. The PL spectrum of PCN is deconvoluted into four emission peaks at 434 (PL1), 451 (PL2), 478, and 512 nm (Figure 4B). The emission peak at the lower wavelength of 434 nm is ascribed to the intrinsic LUMO to HOMO charge transfer whereas an intense peak at 451 nm arises from the band-to-band recombination of photoexcitons. The other two low-intensity peaks at 478 and 512 nm are ascribed to the defect recombination of the other two emissions. On the other hand, the PL emission spectra for NPCN is deconvoluted into five peaks at 406, 434 (PL1), 450 (PL2), 479, and 511 nm (Figure 4C). Of these five emission peaks, four peaks at 434, 450, 479, and 511 nm are consistent with that of PCN, attributed to the transitions mentioned above. Remarkably, an additional hybridized peak at 406 nm was observed for NPCN, which might arise from the near-band gap emission or intermediate trap states. Intermediate states arise mainly because of the dopant molecules such as excessive nitrogen atoms. This new hybrid mid-gap state is advantageous for the facile transport of photogenerated charge carriers. Hence, the modified band structure of NPCN can potentially accelerate the charge transfer rate and enhance photoactivity.

To better comprehend the photoexcitons recombination kinetics, time-resolved photoluminescence (TR-PL) characteristics were investigated (Figure 4D). The exponential decay kinetics fitting results of all three catalysts are summarized in Table S4. The fitted components with the time constant τ1, τ2, and τ3 represent the recombination of photogenerated electrons from σ*, π*, and intermediate trap states to the ground state, respectively. The average lifetime thus calculated after fitting is found to be 1.7, 0.6,
and 0.8 ns for PCN, APCN, and NPCN, respectively. The two decay components, $t_1$ and $t_2$ of NPCN is found to be smaller in comparison to PCN. This indicates better charge mobility in NPCN catalysts. Furthermore, a remarkable increase in the magnitude of $t_3$ is observed in the case of NPCN indicates the trapping of photo-generated electrons in the intermediate trap states. Conclusively, electrons are excited to the conduction band on light irradiation, and holes are generated in the valence band of the respective PCN catalyst. The photoexcited electrons in NPCN, while returning to the ground state, get trapped in the intermediate trap states. These trapped electrons likely to participate in photocatalytic reactions thereby increasing the photocatalytic performance of the NPCN catalyst. The efficient separation of electron-hole pairs in synthesized catalyst is further evaluated by electrochemical impedance spectroscopy (EIS). The EIS Nyquist plot demonstrated a smaller arc diameter for NPCN catalyst than PCN and APCN catalysts (Figure S15). Evidently, the smaller arc diameter for NPCN indicates a low charge transfer resistance and an efficient separation of charge carriers. This result further suggests that NPCN catalyst show efficient separation of photoexcitons.

In addition, the 2',7'-dichloro dihydro fluorescein diacetate (DCFH-DA) assay was used to fluorescently estimate the amount of reactive oxidative species (ROS) generated by PCN, APCN, and NPCN. DCFH-DA was first hydrolyzed to 2',7'-dichlorodihydrofluorescein (DCFH) by incubating it with 100 μL of KOH (1 M) for 5 min then diluted into 10 μL concentration. In a typical experiment, DCFH (200 μL) was incubated with different materials (2 mg/mL) for 4 h at 37°C, and the fluorescence was measured at excitation/emission wavelengths of 485/535 nm, respectively. From the fluorescence emission spectrum, the highest fluorescence emission intensity was observed for NPCN relative to PCN and APCN (Figure S16). This indicates that NPCN generates more ROS on illumination.

The surface area of catalysts powders is measured by nitrogen adsorption-desorption isotherm illustrated in Figure 4E, and the corresponding surface area, pore size, and pore volume are tabulated in Table 2. The specific surface areas of PCN, APCN, and NPCN are measured as 13.0, 21.9, and 129.1 m²g⁻¹, respectively. The NPCN catalyst shown almost ten-fold increment in specific surface area than PCN and APCN. Furthermore, NPCN also shown a high pore volume of 0.190 cm³g⁻¹, much higher than the PCN (0.019 cm³g⁻¹) and APCN (0.033 cm³g⁻¹) counterparts (Figure 4F). The considerable increase in surface characteristics of NPCN suggests the creation of a nanoporous structure, which was most likely caused by the release of excess CO₂, NH₃, and HCl during the thermal polymerization process of the nitrogen rich AGH additive. It is evident that increasing the number of active sites on the catalyst surface improves adsorption of reactants and accelerates the photocatalytic process. In nutshell, the outstanding photocatalytic performance of NPCN shown here can be related to the abundance of high surface-active sites.

### Table 2. Textural parameters of the catalysts

| Sample | Surface area (m²g⁻¹) | Pore radius (nm) | Pore volume (cm³g⁻¹) |
|--------|----------------------|------------------|----------------------|
| PCN    | 13.0                 | 1.06             | 0.019                |
| APCN   | 21.9                 | 1.41             | 0.033                |
| NPCN   | 129.1                | 1.42             | 0.190                |

Theoretical insights using density functional theory calculations

Density-functional theory (DFT) calculations were performed to efficiently grasp the influence of N-doping on the electronic structure characteristics of PCNs. The optimized structures and corresponding density of states (DOS) of PCN, APCN, and NPCN structures are shown in Figures 5A, 5B, and S17. The incorporation of nitrogen displays marked variations in the electronic properties of PCN. To note, a new intermediate bandgap state (mid-gap electronic state), was identified at 0.91 eV and 0.84 eV above the valence band maxima in APCN and NPCN band structure, respectively. The mid-gap electronic state is mainly composed of the doped nitrogen atoms, which suggests that doped nitrogen atoms incorporate mid-gap electronic states in NPCN catalyst. This mid-gap electronic state can positively accelerate the separation of charge carriers and uplift photoactivity. These theoretical results are consistent with the PL observations (Figure 4A). Henceforth, the enhanced photoinduced charge carrier separation in APCN and NPCN can be attributed to the nitrogen atom induced mid-gap energy levels that appeared above the Fermi level in the forbidden band. Under light excitation, the electrons present in the intermediate energy levels can transfer to the conduction band, which increases the generation and...
separation efficiency of photoinduced carriers and thereby promotes photocatalytic activity. It should also be noted that the defect levels may act as a recombination centre to trap electrons and thereby shortening the lifetime of unbalanced carriers.\(^{40}\) In addition, the charge transfer mechanism between hydrogen and pure/doped PCN systems is analyzed using charge density difference (\(\Delta \rho\)) (Equation 2);

\[
\Delta \rho = \rho_{\text{Cat}+H} - \rho_{\text{Cat}} - \rho_{\text{H}} \tag{Equation 2}
\]

where, \(\rho_{\text{Cat}+H}, \rho_{\text{Cat}},\) and \(\rho_{\text{H}}\) represents the charge density of hydrogen adsorbed catalyst, pristine catalyst, and H atom, respectively. The computed charge density differences are shown in Figure 5C, where the blue and green colors indicate electron accumulation and depletion regions, respectively. In the case of PCN, the adsorption of hydrogen leads to a slight electron transfer from the surface to hydrogen whereas the introduction of N dopants increases the transfer of electrons, which is responsible for the enhancement of HER activity. Typically, the Gibbs free energy (\(\Delta G_{\text{H}^+}\)) is the universal descriptor of the HER activity, where the optimal \(\Delta G_{\text{H}^+}\) (close to zero) can make the H\(^+\) adsorption/desorption spontaneous without an activation barrier.\(^{41}\) The \(\Delta G_{\text{H}^+}\) versus reaction coordinate of the HER for the studied systems were shown in Figure 5D.

The pristine PCN is found to be inert for HER with a relatively large \(\Delta G_{\text{H}^+}\) of \(-0.49\) eV. The introduction of doping notably decreased \(\Delta G_{\text{H}^+}\) to \(-0.21\) and \(-0.18\) eV for APCN and NPCN, respectively, showing a significant improvement over PCN. This demonstrates that the \(\Delta G_{\text{H}^+}\) of PCN can be efficiently manipulated by doping nitrogen atoms to attain the optimal HER activity.

Unravelling the underlying catalytic mechanism for enhanced photoactivity

To learn more about the NADH photooxidation reaction mechanism on the surface of NPCN, quenching experiments were performed with various scavengers. The photooxidation efficiency severely decreased to 64% and 35% on using isopropanol (IPA) and \(p\)-benzoquinone (\(p\)-BQ) as hydroxyl (OH\(^-\)) and superoxide...
radical (O$_2^-$) scavengers, respectively (Figure S18). Although CuCl$_2$ (to trap holes) and KI (to trap electrons) did not affect photooxidation activity. This confirms that OH$^-$ and O$_2^-$ synergistically oxidize NADH. The CIP degradation performance significantly dropped from 76% to 40%, and 23% using ammonium oxalate (AO as hole scavenger), and p-BQ, respectively (Figure S19). The dye degradation performance also shown a significant reduction in the degradation performance in the presence of p-BQ and AO, which indicates O$_2^-$, and h$^+$ both carry out degradation of CIP and RhB (Figure S20). Although 64% CIP degradation in the presence of IPA indicates a minor role of hydroxyl radicals, the dye degradation performance remains unaltered in presence of IPA. This proves that hydroxyl radicals have minor role in dye degradation reaction over NPCN catalyst.

Furthermore, to confirm the feasibility of OH$^-$ and O$_2^-$ formation on NPCN, the conduction band, and valence band positions were calculated by Mulliken electronegativity equation (refer STAR Methods for details). The corresponding VB and conduction band CB positions of NPCN were calculated to be +1.72 and −0.99 eV (vs. NHE), respectively. Meanwhile, in NPCN, the E$_g$ obtained from the K-M plot is 2.47 eV. As a result, the position of mid-gap state is calculated to be −0.75 eV (vs. NHE). The optimum potential required to generate O$_2^-$ (O$_2$/O$_2^-$) and OH$^-$ (OH$^-$/OH$^-$) is −0.046 and +2.3 eV versus NHE, respectively. Perhaps, the CB potential of NPCN is much lower than the standard oxidation potential, and hence generation of O$_2^-$ by photogenerated electrons is feasible. On the other hand, the valence band potential of NPCN is lower than the required potential for the formation of OH$^-$. Thus, the photogenerated holes cannot directly oxidize OH$^-$ to OH. But the quenching experiments revealed a significant contribution of OH$^-$. This suggests the indirect generation of OH$^-$ from O$_2^-$ via H$_2$O$_2$ generation in the aqueous medium.

To support the generation of various ROS after light irradiation, ESR trapping experiments were conducted. The ESR spectrum with 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) as spin trapping agent exhibits ESR signals with an intensity ratio of 1:2:2:1 attributed to the DMPO-OH$^-$, which confirms the generation of OH$^-$ (Figure S21A). In this context, high-intensity sharp signals were observed with NPCN catalyst in comparison to PCN. This indicates that the NPCN catalyst is more capable of generating OH$^-$ under illumination. The generation of superoxide radicals on illumination is further detected by ESR spin trapping by the addition of solvent DMSO with DMPO. On illumination, a twelve-line signal corresponding to DMPO-O$_2^-$ / OOH adduct was generated (O$_2^-$ receives H$^+$ in DMSO to generate DMPO-O$_2^-$ / OOH adduct) (Figure S21B). Furthermore, ESR signals detected are assigned to DMPO-CH$_3$ and DMPO-OCH$_3$ reflecting the interaction of OH$^-$ with DMSO, and oxygen and their trapping by DMPO. $^{42-45}$ The ESR trapping results favor the generation of OH$^-$ and O$_2^-$ on NPCN catalyst.

Based on the scavenger’s experiment, a plausible photooxidation mechanism is proposed in Figure S21C. Accordingly, on light illumination, NPCN generates electrons and holes in the CB and VB, respectively. During relaxation, electrons get trapped in the mid-gap electronic state of NPCN. These trapped electrons then further participate in the catalytic reaction. The photogenerated holes first oxidize NADH to the corresponding cationic radical (NADH$^+$) and electrons generate superoxide radicals on reaction with dissolved oxygen. The superoxide radicals later abstract protons from NADH$^+$ to generate NAD$^+$.$^+$ Meanwhile, superoxide radical gets reduced to hydroperoxyl radical (OOH$^-$), which subsequently leads to the generate hydroxyl radicals (OH) via peroxide formation. The hydroxyl radicals continue abstracting protons from NADH$^+$ to generate NAD$^+$. Nevertheless, the VB of the catalyst NPCN is not enough positive to generate OH$^-$ from water molecules and holes. Hence, the hydroxyl radical is produced from superoxide radical via H$_2$O$_2$ formation.

As discussed in the above section, after illuminating NPCN catalyst, electrons and holes are generated in the CB and VB of NPCN catalyst. The excited electrons are then transferred to the mid-gap states, which is located amid CB and VB at −0.75 eV. The two-electron reduction of protons to hydrogen requires a potential of H$^+$/H$_2$ = 0 eV. The CB as well as mid-gap energy states are located above the reduction potential of protons. Hence, NPCN catalyst can generate hydrogen. The electrons in mid-gap states react with protons in the aqueous medium to generate H$_2$ as shown in Scheme 1. Furthermore, the quenching results suggests that O$_2^-$ and holes are plays important role in degradation process of antibiotic CIP and dye RhB. The electrons in the mid-gap state of NPCN catalyst generates O$_2^-$, which oxidizes and mineralize CIP and RhB into CO$_2$ and H$_2$O, whereas the holes in the VB of NPCN degrades CIP and RhB as shown in the Scheme 1.
In summary, we employed an additive-assisted approach to synthesize a nitrogen-rich carbon nitride (NPCN) photocatalyst. The incorporation of additional nitrogen atoms into the heptazine network of PCN imparts mid-gap electronic states within the band gap and decreases the C/N ratio to 0.70, modifying its band structure. Besides N doping, the specific surface area of NPCN catalyst increased to 129.1 m²g⁻¹. As a result, the N-rich NPCN photocatalyst exhibited excellent NAD⁺ regeneration ability, owing to the improved charge transfer dynamics and high surface-active sites. The NPCN also demonstrated a high H₂ evolution rate of 11.18 mmolg⁻¹h⁻¹ with AQY of 9.16% at λ: 420 nm and rapid degradation kinetics for antibiotic ciprofloxacin and rhodamine B dye. The N-rich NPCN type catalyst shown here represents an important conceptual advance in designing of high-performance metal-free photocatalyst through a simple additive engineering approach. The findings presented in this work can encourage the further development of efficient additive based PCN like photocatalysts for energy and environmental cleaning applications.

Limitations of the study
This study reports additive assisted synthesis of N-rich PCN photocatalyst for NADH oxidation, hydrogen production, and degradation of pollutants. Because photocatalytic NADH oxidation is technically feasible, studies towards concurrent photo-enzymatic product conversion will add significant value to this approach. In addition, photodegradation of pollutants under actual wastewater circumstances is necessary to investigate the efficacy of the photocatalyst.

STAR METHODS
Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION
Supplemental information can be found online at https://doi.org/10.1016/j.isci.2022.105567.

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AUTHOR CONTRIBUTIONS
Conceptualization, T.B. and V.D.; Methodology, T.B. and D.V.; Investigation, T.B., D.J.K., A.G., B.M.A., N.M., N.R.M., S.T., D.V., and S.S.U.; Resources, S.S.U; Formal Analysis, T.B., D.J.K., A.G., B.M.A., N.M., S.T., and D.V.; Visualization, T.B., D.V., and S.S.U.; Writing – Original Draft, T.B.; Writing – Review and Editing, B.M.A., N.R.M., S.T., V.D., and S.S.U.; Supervision, D.V. and S.S.U., Funding Acquisition, S.S.U.

DECLARATION OF INTERESTS
The authors declare no competing interests.

INCLUSION AND DIVERSITY
We support inclusive, diverse, and equitable conduct of research.

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REFERENCES
1. Zhang, S., Shi, J., Chen, Y., Huo, Q., Li, W., Wu, Y., Sun, Y., Zhang, Y., Wang, X., and Jiang, Z. (2020). Unraveling and manipulating of NADH oxidation by photogenerated holes. ACS Catal. 10, 4967–4972.
2. Schomburg, D., Schomburg, I., and Chang, A. (2013). Class 1: Oxidoreductases: EC 1 (Springer).
3. Fujita, S., Matsumoto, R., Ogawa, K., Sakai, H., Maesaka, A., Tokita, Y., Tsujimura, S., Shirai, O., and Kano, K. (2013). Bioelectrocatalytic oxidation of glucose with antibiotic channel-containing liposomes. Phys. Chem. Chem. Phys. 15, 2650–2653. https://doi.org/10.1039/C3CP4998D.
4. Nishigaki, J.-i., Ishida, T., Honma, T., and Haruta, M. (2020). Oxidation of β-nicotinamide adenine dinucleotide (NADH) by Au cluster and nanoparticle catalysts aiming for coenzyme regeneration in enzymatic glucose oxidation. ACS Sustain. Chem. Eng. 8, 10413–10422. https://doi.org/10.1021/acssuschemeng.0c01893.
5. Zhang, Y., Liu, X., Li, Y.Q., Tang, L.L., Chen, L., Ma, J., and Liu, J. (2020). Iron-doping accelerating NADH oxidation over carbon nitride. Front. Oncol. 10, 1076–1082.
6. Ryu, G.M., Lee, M., Choi, D.S., and Park, C.B. (2015). A hematite-based photoelectrochemical platform for visible light-induced biosensing. J. Mater. Chem. B 3, 4483–4486. https://doi.org/10.1039/C5TB00478K.
7. Samantara, A.K., Chandra Sahu, S., Bag, B., Jena, B., and Jena, B.K. (2014). Photoelectrocatalytic oxidation of NADH by visible light driven plasmonic nanocomposites. J. Mater. Chem. A Mater. 2, 12677–12680. https://doi.org/10.1039/C4TA01349B.
8. Ma, B.C., Caire da Silva, L., Jo, S., Wurm, F.R., Bannwarth, M.B., Zhang, K.A.I., Sundmacher, K., and Landfester, K. (2019). Polymer-based module for NAD+ regeneration with visible light. Chembiochem 20, 2593–2596.
9. Byun, J., Landfester, K., and Zhang, K.A.I. (2019). Conjugated polymer hydrogel photocatalysts with expandable photoactive sites in water. Chem. Mater. 31, 3381–3387.
10. Ong, W.J., Tan, L.L., Ng, Y.H., Yong, S.T., and Chai, S.P. (2016). Graphitic carbon nitride (g-C3N4)-based photocatalysts for artificial photosynthesis and environmental remediation: are we a step closer to achieving sustainability? Chem. Rev. 116, 7159–7329. https://doi.org/10.1021/acs.chemrev.6b00075.
11. Guo, S., Deng, Z., Li, M., Jiang, B., Tian, C., Pan, Q., and Fu, H. (2016). Phosphorus-doped carbon nitride tubes with a layered micro-nanostructure for enhanced visible-light photocatalytic hydrogen evolution. Angew. Chem. Int. Ed. Engl. 128, 1862–1866.
12. Wang, Y., Phua, S.Z.F., Dong, G., Liu, X., He, B., Zhai, Q., Li, Y., Zheng, C., Quan, H., Li, Z., and Zhao, Y. (2019). Structure tuning of polymeric carbon nitride for solar energy conversion: from nano to molecular scale. Chem 5, 2775–2813. https://doi.org/10.1016/j.chempr.2019.07.019.
13. Guo, S., Tang, Y., Xie, Y., Tian, C., Feng, Q., Zhou, W., and Jiang, B. (2017). P-doped tubular g-C3N4 with surface carbon defects: universal synthesis and enhanced visible-light photocatalytic hydrogen production. Appl. Catal. B Environ. 218, 664–671. https://doi.org/10.1016/j.apcatb.2017.07.022.
14. Jing, L., Wang, D., Xu, Y., Xie, M., Yan, J., He, M., Song, Z., Xu, H., and Li, H. (2020). Porous defective carbon nitride obtained by a universal method for photocatalytic hydrogen production from water splitting. J. Colloid Interface Sci. 566, 171–182.
21. Vidyasagar, D., Bhoyar, T., Singh, G., and Umare, S.S. (2022). Tailoring photocactivity of polymeric carbon nitride via donor-π-acceptor network. Appl. Catal. B Environ. 310, 121347.

26. Kar, S., Bishwal, L., Kommula, B., and Bhattacharya, S. (2022). Critical optimization of phosphorus functionalized carbon nanomaterials for metal-free solar hydrogen production and simultaneous organic transformation. Adv. Optic. Mater. 2102641. https://doi.org/10.1002/adom.202102641.

27. Wei, Y., Li, Y., Liu, Q., Zhang, Y., Zhang, K., Huo, P., and Yan, Y. (2022). Leaf-Vein structure like g-C3N4/P-MWNTs donor-acceptor hybrid catalyst for efficient CO2 photoreduction. Carbon 188, 59–69.

28. Bhoiyar, T., Vidyasagar, D., and Umare, S.S. (2023). Mitigating phototoxicity of tetracycline metal-free free-base pyrroloquinoline functionalized carbon nitride photocatalyst. J. Environ. Sci. 125, 37–46. https://doi.org/10.1016/j.jes.2021.10.032.

29. Wu, B., Zhang, L., Ji, Y., Tian, C., Li, S., and Guo, X. (2021). Self-supporting 3D carbon nitride with tunable n → m* electronic transition for enhanced solar hydrogen production. Adv. Mater. 33, 2104361. https://doi.org/10.1002/adma.202104361.

30. An, S., Zhang, G., Li, K., Huang, Z., Wang, X., Guo, Y., Hou, J., Song, C., and Guo, X. (2021). Porous carbon nitride thin strip: -electron induces elevated photocatalytic hydrogen evolution. Carbon 153, 757–766.

31. Wang, X., Maeda, K., Thomas, A., Takahanbe, K., Xin, G., Carlson, J.M., Domen, K., and Antonietti, M. (2009). A metal-free polymeric photocatalyst for hydrogen production from water under visible light. Nat. Mater. 8, 76–80. https://doi.org/10.1038/nmat2317.

32. Li, J., Wang, X., Huang, L., Tian, L., Shalom, M., Xiong, C., Zhang, H., Jia, Q., Zhang, S., and Liang, F. (2021). Ultrathin mesoporous-graphitic carbon nitride nanosheets with functional cyano group decoration and nitrogen-vacancy defects for an efficient selective CO2 photoreduction. Nanoscale 13, 12634–12641.

33. Piree, S., Waseed, S., Sharma, P., and Karak, S. (2020). Structural and optical properties of exfoliated graphene-like carbon nitride into nanosheets and quantum dots. Mater. Char. 169, 110646. https://doi.org/10.1016/j.matchar.2020.110646.

34. Dong, F., Li, Y., Wang, Z., and Ho, W-K. (2015). Enhanced visible light photocatalytic activity and oxidation ability of porous graphene-like g-C3N4 nanosheets via thermal exfoliation. Appl. Surf. Sci. 358, 393–403.

35. Huang, X., El-Sayed, H., Yi, X., and El-Sayed, M.A. (2005). Gold nanoparticles: catalyst for the oxidation of NADH to NAD+. J. Photochem. Photobiol. B 81, 76–83. https://doi.org/10.1016/j.jphotobiol.2005.05.010.

36. Ghorui, U.K., Satra, J., Mondal, P., Mardanya, S., Sarkar, A., Srivastava, D.N., Adhikary, B., and Mondal, A. (2021). Graphitic carbon nitride embedded Ag nanocube decorated-ZnWO4 nanocomposite-based photoluminescence sensing of Hg2+. Mater. Adv. 2, 4041–4057.
45. Tang, S., Liu, Z., Ma, F., Cao, G., Wang, J., Chen, W., Feng, X., and Zhu, Y. (2020). Catalytic activity of porous carbon nitride regulated by polyoxometalates under visible light. RSC Adv. 10, 8255–8260.

46. Lu, N., Yin, D., Li, Z., and Yang, J. (2011). Structure of graphene oxide: thermodynamics versus kinetics. J. Phys. Chem. C 115, 11991–11995.

47. Kresse, G., and Furthmüller, J. (1996). Efficient iterative schemes for ab initio total-energy calculations using a plane-wave basis set. Phys. Rev. B Condens. Matter 54, 11169–11186.

48. Blochl, P. E. (1994). Projector augmented-wave method. Phys. Rev. B 50, 17953–17979.

49. Perdew, J. P., Burke, K., and Ernzerhof, M. (1996). Generalized gradient approximation made simple. Phys. Rev. Lett. 77, 3865–3868.

50. Perdew, J. P., Ernzerhof, M., and Burke, K. (1996). Rationale for mixing exact exchange with density functional approximations. J. Chem. Phys. 105, 9982–9985.

51. Monkhorst, H. J., and Pack, J. D. (1976). Special points for Brillouin-zone integrations. Phys. Rev. B 13, 5188–5192.

52. Heyd, J., Scuseria, G. E., and Ernzerhof, M. (2003). Hybrid functionals based on a screened Coulomb potential. J. Chem. Phys. 118, 8207–8215.

53. Grimme, S., Antony, J., Ehrlich, S., and Krieg, H. (2010). A consistent and accurate ab initio parametrization of density functional dispersion correction (DFT-D) for the 94 elements H–Pu. J. Chem. Phys. 132, 154104.
**STAR METHODS**

**KEY RESOURCES TABLE**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Other**           |        |            |
| Melamine (99%)      | Sigma Aldrich (USA) | 108-78-1   |
| Urea (99%)          | Merck (India) | 57-13-6    |
| Aminoguanidine hydrochloride (>98) | Tokyo Chemical Industry (TCI, India) | 1937-19-5 |
| β-Nicotinamide Adenine Dinucleotide | Tokyo Chemical Industry (TCI, India) | 606-68-8 |
| Disodium Salt Hydrate (NADH) | | |
| Triethanolamine (>99%) | Sigma Aldrich (USA) | 102-71-6 |
| Potassium iodide (>99) | Merck (India) | 7681-11-0 |
| p-benzoquinone (98%) | Sigma Aldrich (USA) | 106-51-4 |
| Ammonium oxalate (>99%) | Fisher Scientific (India) | 6009-70-7 |
| Copper chloride | Merck (India) | 7447-39-4 |
| Isopropyl Alcohol | Fisher Scientific (India) | 67-63-0 |
| Ciprofloxacin (98%) | Tokyo Chemical Industry (TCI, India) | 85721-33-1 |
| Rhodamine B | British Drug House LTD. (BDH, England) | 676750 |

| Software and algorithms | | |
|-------------------------|-----------------------------|
| Image J | National Institute of Health | [https://imagej.nih.gov/ij/] |
| Origin 2018 | Origin Lab | [https://www.originlab.com/] |
| ChemDraw Professional 15.0 | Perkin Elmer | [https://perkinelmerinformatics.com/products/research/chemdraw] |

**RESOURCE AVAILABILITY**

**Lead contact**

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Suresh S. Umare (ssumare1965@gmail.com).

**Materials availability**

This study did not generate new unique reagents.

**Data and code availability**

All data reported in this paper will be shared by the lead contact upon request.

**METHOD DETAILS**

**Synthesis of catalysts**

The polymeric carbon nitride catalysts were synthesized by annealing precursors at 550°C in a muffle furnace under a static air atmosphere. The bulk polymeric carbon nitride (PCN) was prepared using melamine as a precursor. The AGH-derived carbon nitride was synthesized by pyrolysis of AGH at 550°C for 2 h which is labelled as APCN. The nitrogen rich PCN was synthesized by the mixture of melamine, urea, and AGH. In brief, 20 mg of AGH and 10 g of urea were mixed to obtain a homogeneous mixture. The mixture was then transferred to a silica crucible and a thin layer of melamine (1 g) was applied over the top. The precursor mixture was then annealed at 550°C for 5 h under a static air atmosphere, and the corresponding sample is labelled as N-rich polymeric carbon nitride (NPCN).

**Photocatalytic NADH oxidation**

The photocatalytic NADH oxidation reaction is performed in a photocatalytic reactor equipped with a visible light source of average light intensity of 500 Wm⁻². In detail, 5 mg of catalyst was dispersed in
50 mL 0.1 mM NADH solution. The reaction mixture was then stirred in the dark to attain adsorption-desorption equilibrium. Next, the reaction mixture was subjected to light irradiation and sample aliquots were collected at a fixed interval of 10 mins. The catalysts powder in aliquots was filtered by a 0.45 μm syringe filter before further analysis. The oxidation of NADH to NAD⁺ was monitored by recording absorption spectra at a target wavelength of 340 nm.

**Photocatalytic hydrogen evolution**

The photocatalytic H₂ evolution study was performed according to our previous report.¹⁵ In a typical process, 4 mg of catalyst was added to 40 mL of deionized water and ultrasonicated for 10 mins. Afterward, 10 vol% TEOA was added to the mixture and the reaction mixture was purged with Ar for 20 mins to remove air. 1wt% Pt was used as cocatalyst. The reaction mixture was then irradiation using a 300W Xe lamp with a 420 nm cut-off filter. The H₂ evolution was detected by gas chromatography (GC-2010, Shimadzu) equipped with TCD with He as carrier gas.

**Photocatalytic ciprofloxacin (CIP) and rhodamine B (RhB) degradation**

The ciprofloxacin (CIP) and rhodamine B (RhB) degradation experiments were performed in the same catalytic reactor used for NADH oxidation. For, CIP degradation, 20 mg of catalyst was dispersed in 50 mL 5 mgL⁻¹ aqueous CIP and 1 mmol citric acid was added as hole scavenger. The reaction mixture was illuminated with light after stirring it in dark for 30 min to attain adsorption-desorption equilibrium. In an interval 20 min, 5 mL sample aliquots were collected and filtered through 0.25 μm syringe filter to analyse by UV-Vis spectrophotometer at 256 nm. While, for RhB degradation experiment, 100 mg of catalyst was dispersed in 100 mL 20 mgL⁻¹ RhB solution. After 30 mins of stirring in dark condition, sample aliquots were collected after every 10 min of light irradiation and degradation was monitored using UV-Vis spectrophotometer at 554 nm.

**Characterization of catalysts**

The powder X-ray diffraction (XRD) pattern was recorded on Brucker D8 advance ECO diffractometer (MA, USA) operated at 40 kV and 25 mA using Cu Kα source (λ = 1.5418 Å). Fourier transforms RAMAN spectrum (FT-RAMAN) was recorded on a Bruker RFS 27 MultiRAM FT RAMAN spectrometer (MA, USA) in a spectral range of 4000–50 cm⁻¹. Fourier transform infrared (FT-IR) spectrum was recorded on Nicolet iS50 FTIR spectrometer (Thermo Fisher Scientific, MA, USA) with KBr pellets in the range 4000–400 cm⁻¹. Cross polarized magic angle spinning (CP-MAS)¹³C NMR spectrum was recorded on ECX400 (JEOL, Tokyo, Japan). An X-ray photoelectron spectroscopy (XPS) ESCALAB 250-Xi (Thermo Fisher Scientific, MA, USA) was used for elemental analysis. Solution state ¹H NMR of the samples was obtained using Bruker AV400 (MA, USA). The optical absorption of the solid materials was measured by ultraviolet diffuse reflectance spectrophotometer (UV-DRS, UV-2600, Shimadzu, Japan). The microstructure of the materials was analysed by transmission electron microscope (TEM) JEM-2100 (JEOL, Tokyo, Japan) with an accelerating voltage of 200 kV. Atomic force microscope (AFM) measurements were carried out using a Bio FastScan scanning probe microscope (Bruker AXS, MA, USA). The absorbance of the liquid samples was measured by an ultraviolet-visible (UV-Vis) spectrophotometer (UV-1800, Shimadzu, Japan). The Brunner Emmette Teller (BET) surface area was measured using nova touch surface area analyzer NT1-1 (Quantachrome, FL, USA). Steady state photoluminescence (PL) emission spectra were recorded on Fluorocube-Life Time system JOBINVYON M/S (NJ, USA). The X-band electron paramagnetic resonance (EPR) was recorded on ESR spectrophotometer Bruker ELEXYSYSR500CW (MA, USA).

**Computational details**

DFT calculations were performed using plane-wave basis set through Vienna Ab Initio Simulation Package (VASP)⁴⁶,⁴⁷ where the ion-electron interactions were described via projector augmented wave (PAW) method.⁴⁸ Within a generalized gradient approximation (GGA-PBE), the electron-electron exchange correlation was described using the Perdew, Burke, and Ernzerhof functional.⁴⁹,⁵⁰ A converged cutoff was set to 500 eV for expanding the wavefunctions over the plane-wave basis set. During structural relaxation, the atomic positions were optimized until the maximum force were reached to 0.01 eVÅ and the energy convergence criteria for ionic and electronic iterations were set to 1 × 10⁻⁵ eV. The Brillouin zone
integration was conducted at $5 \times 5 \times 1$ and $9 \times 9 \times 1$ Monkhorst-Pack k-point mesh for structural optimization and electronic properties, respectively. To avoid the interaction between the periodic images, a vacuum space of 15 Å was introduced along the z direction. To obtain accurate band gap values, the HSE06 hybrid functional was adopted with 0.25 mixing parameter ($\alpha$). The DFT-D3 dispersion correction proposed by Grimme is considered to incorporate the van der Waals (vdW) correction originating from the interaction of electric dipole moments.

The Gibbs free energy ($\Delta G_{\text{H}}$) associated with adsorption of H is a better descriptor to understand the HER performance, which can be defined as:

$$\Delta G_{\text{H}} = \Delta E - T\Delta S + \Delta E_{\text{ZPE}}$$  \hspace{1cm} \text{(Equation 3)}

Where $\Delta E$ is the DFT simulated reaction energy. $T$ is the temperature ($T = 298.15$ K). $\Delta S$ is the difference of vibration entropy between gas phase hydrogen and adsorbed hydrogen. $\Delta E_{\text{ZPE}}$ is the zero-point energy corrections.

**Apparent quantum yield (AQY) calculation**

The apparent quantum yield (AQY) of NPCN was determined at $\lambda \geq 420$ nm using a 420 nm band pass filter (20BP10-420, Newport). The AQY was calculated from the Equation 4;

$$\text{AQY} = \frac{\text{number of reacted electrons}}{\text{number of incident photons}} \times 100\%$$  \hspace{1cm} \text{(Equation 4)}

$$= \frac{\text{number of evolved hydrogen molecules} \times 2}{\text{number of incident photons}} \times 100\%$$

$$= \frac{2 \times N_A \times n_{\text{H}_2}}{I \times S \times \frac{l}{c} \times \lambda} \times 100\%$$

Where $N_A$, $n_{\text{H}_2}$, $I$, $S$, $\lambda$, $h$, and $c$ are Avogadro’s number, hydrogen molecules evolved in time $t$, intensity of incident light, irradiation area, wavelength, planck’s constant and velocity of light, respectively. The intensity of light source ($I$) was recorded to be 1.5 mWcm$^{-2}$ and the irradiation area ($S$) was 5.73 cm$^2$. After 3 h of irradiation, NPCN catalyst generated 14.92 µmol of hydrogen at $\lambda \geq 420$ nm.

$$\text{AQY} = \frac{2 \times 6.02 \times 10^{23} \text{[mol$^{-1}$]} \times 14.92 \times 10^{-6} \text{[mol]} \times 1.5 \times 10^{-3} \text{[W$\cdot$m$^{-2}$]} \times 5.73 \times 10^{-3} \text{[m$^2$]} \times 10800 \text{[s]} \times \frac{4.2 \times 10^{-7} \text{[J]} \times 3 \times 10^8 \text{[m$^2$]$\cdot$s$^{-1}$]}{6.63 \times 10^{-34} \text{[J$\cdot$m$^2$]$\cdot$s]} \times 3 \times 10^3 \text{[m$^2$]$\cdot$s$^{-1}$]}} \times 100\%$$

$$= 9.16\%$$

**Estimation of band edge potential**

To evaluate the catalytic reaction mechanism over defective NPCN, the band edge positions were calculated using Mulliken electronegativity equation as follow;

$$E_{\text{CB}} = \chi - 0.5E_g - E_x$$  \hspace{1cm} \text{(Equation 5)}

$$E_{\text{VB}} = E_{\text{CB}} + E_g$$  \hspace{1cm} \text{(Equation 6)}

where, $E_{\text{CB}}$ and $E_{\text{VB}}$, denotes the conduction band (CB) and valence band (VB) potentials in eV respectively; $\chi$ represents absolute electronegativity, $E_x$ is the energy of free electrons ($E_x = 4.5$ eV vs. NHE), and $E_g$ is the bandgap of the semiconductor catalyst. The CB and VB potentials are calculated to be $-0.99$ and $+1.72$ eV, respectively.