The Evolution of Hydrogen Production Catalysts from Enzymes to Artificial Bio-Hybrid Systems

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Abstract

The production of hydrogen, a clean and sustainable energy carrier, has been a major focus of research in recent decades. Hydrogenase enzymes, have long set the benchmark for efficiency in hydrogen production, catalyzing the reversible conversion of protons to molecular hydrogen with a remarkable speed and minimal overpotential requirement. However, their sensitivity to oxygen and instability beyond their native environment present significant challenges for their direct practical applications. This review explores the journey from natural hydrogenase enzymes to synthetic and bio-hybrid systems designed to understand its mechanism and overcome these limitations. We trace the evolution of hydrogen production technologies, beginning with the mimicry of hydrogenase active sites using small-molecule catalysts and progressing to the incorporation of these catalysts into protein frameworks. The review discusses key challenges, including sensitivity, scalability, integration into devices, and highlights future perspectives, such as the development of bio-inspired catalysts and their potential in creating next-generation hydrogen production systems. By examining these developments, we underscore the progress made in strategic replicating and surpassing the efficiency of natural hydrogenases, paving the way for more robust and scalable hydrogen production technologies for a greener future.

Keywords: Electrocatalytic Hydrogen Production, Hydrogenase, Biomimetic Catalyst Design, Artificial Metalloprotein, Metallopeptide.

Introduction

The global pursuit of sustainable energy solutions has positioned hydrogen as a critical component in the transition to a low-carbon economy and future energy systems.[1] As an energy carrier, hydrogen offers a clean and versatile solution, capable of powering a wide range of applications, from transportation to industrial processes.[2] However, the efficient and environmentally friendly scalable production of hydrogen remains a formidable challenge, particularly in a world seeking to reduce its reliance on fossil fuels.[3,4] In nature, the enzyme Hydrogenase provides an elegant and efficient route for hydrogen production, catalyzing the reversible conversion of protons to hydrogen under ambient conditions.[5] Hydrogenase enzymes, primarily found in microorganisms, have evolved to perform this task

with extraordinary catalytic efficiency, making them a benchmark for hydrogen production (**Fig. 1**). These enzymes operate with high turnover frequencies and minimal overpotential, setting a standard that synthetic systems strive to achieve.[6] However, the existing technologies, such as photoelectrochemical cells, electrolyzers, and proton-exchange-membrane fuel cells (PEMFC), utilizes platinum (Pt) or palladium (Pd)-based materials due to its exceptional catalytic abilities.[7] Interestingly, hydrogenases have emerged as a potential rival to state-of-the-art catalyst Pt, while utilizing only first-row transition metals, specifically nickel and/or iron, at their active sites.[8,9] This makes them a promising alternative to expensive and sparsely available platinum in the green hydrogen technologies. Despite their impressive natural efficiency, hydrogenase enzymes face significant challenges when considered for practical applications. One of the major issues is their inherent sensitivity to oxygen, which can irreversibly inactivate the enzyme. This oxygen sensitivity limits their use in environments where oxygen is present, such as in industrial settings or in open-air conditions.[6,10–12] Additionally, the stability of hydrogenase enzymes outside their native biological contexts is often compromised, making it difficult to integrate them into engineered systems.[13,14] The complexity of these enzymes also poses challenges for large-scale production and integration into devices like electrolyzers, where stability, scalability, and cost are critical parameters. These challenges have spurred extensive research into mimicking the function of hydrogenase through synthetic catalysts, proteins, peptides and bio-hybrids.[15,16] By replicating the active site of hydrogenase using small molecular complexes, researchers aim to capture the enzyme's catalytic prowess while overcoming its limitations. This biomimetic approach has led to the development of an array of synthetic catalysts that, while promising, often fall short of replicating the full efficiency and robustness of natural hydrogenase.[17,18] In parallel, there has been a growing interest in exploring proteins and peptides as alternative platforms for hydrogen production. These biological scaffolds [also defined as the outer coordination sphere (OCS)], offer unique advantages, including biocompatibility, structural versatility, and the potential for fine-tuning catalytic environments. By incorporating molecular catalysts into proteins, researchers have been able to create bio-hybrid systems that combine the catalytic strength of synthetic complexes with the sophisticated structural control inherent to biological molecules.[19,20] This approach represents a significant advancement, moving closer to the goal of replicating the efficiency of natural hydrogenase in a stable and scalable form. This review traces the evolution of hydrogen production strategies, beginning with the pioneering studies on hydrogenase enzymes and extending to the most recent developments in protein- and peptide-based systems. We will explore the key milestones and innovations in the field, culminating in a unique future direction, where we discussed the possibility of integrating a molecular catalyst into stable protein scaffolds and utilize them for H_2 production in electrolyzer assembly. This review not only addresses some of the fundamental challenges associated with hydrogenase but also opens new possibilities for sustainable hydrogen Productions

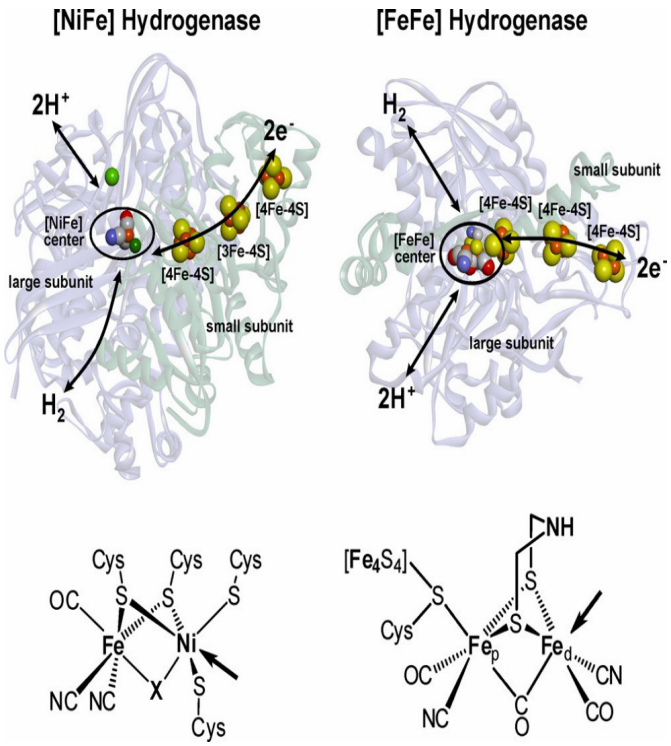


Figure 1. The structures of the [NiFe] hydrogenase from *DvMF*[21] and the [FeFe] hydrogenase from *Dd*[22] are depicted, along with schematic representations of the electron transfer (ET) chain via iron–sulfur centers, as well as pathways for dihydrogen (H_2) and proton (H^+) transfer. At the bottom, the chemical structures of the active sites for both types of hydrogenases are shown, with arrows indicating the open metal coordination sites. The figure is taken from ref [6], copyright ACS [2014].

Hydrogenase Enzymes: Nature's Own H_2 production Unit

The study of certain organisms, including bacteria, archaea, and algae, has led to the remarkable discovery that hydrogen can be both produced and utilized as a source of low-potential electrons within living cells, playing a role in a global hydrogen cycle.[23] A notable example is the bacterium *Ralstonia eutropha*, a chemolithoautotrophic organism, that uses hydrogen as its sole energy source.[24] Similarly, microalgae like *Chlamydomonas reinhardtii*, under specific conditions, can harness sunlight to reverse the typical reaction, i.e. reducing H^+ into H_2 by extracting electrons from water.[24,25] This remarkable biochemical process is enabled by metalloenzymes known as *Hydrogenases*.[24,26] These metalloenzymes are a diverse group that catalyze one of the simplest molecular reactions: the conversion of dihydrogen into protons and electrons, as well as the reverse process, the generation of dihydrogen.[27]

$$H_2 (\rightleftharpoons H^+ + H^-) \rightleftharpoons 2H^+ + 2e^- \quad \textbf{(Equation 1)}$$

This reaction occurs at a specialized metal center, which significantly enhances the acidity of H_2, leading to its heterolytic splitting—a process strongly accelerated by the presence of a nearby base. The reverse reaction, the synthesis of H_2, involves the coupling of H^+ and H^-. Based on the metal ions in their active sites, hydrogenases are categorized as [FeFe] and [NiFe] hydrogenases **(Fig.1)**, which perform the catalysis of these reactions at minimal overpotential and at remarkably high rates, converting 1,500 to 20,000 molecules of H_2 per second at 37°C in neutral water.[28–30] The third type, [Fe]-hydrogenase, also known as Hydrogen-forming methylene-tetrahydromethanopterin dehydrogenase (Hmd), is unique to methanogenic archaea, where it activates H_2 only in the presence of a hydride (H^-) acceptor/donor substrate necessary for the reaction.[31] [FeFe]-hydrogenases are distinguished by a sophisticated active site known as the H-cluster, which comprises a [2Fe] subcluster or diiron center connected to a [4Fe-4S] cubane cluster.[22,32] The diiron center is coordinated by carbon monoxide (CO) and cyanide (CN^-) ligands which was first detected by FTIR spectroscopy, are essential for catalysis.[33,34] These

ligands act as strong π-accepting ligands to iron, facilitating metal-to-ligand back bonding resulting in the stabilization of the low oxidation states of iron. Additionally, several hydrogen bonds to the CN^- ligands help stabilize a specific orientation of the diiron subcluster within the active site.[35,36] The [4Fe-4S] cluster plays a critical role in electron transfer, enabling the efficient reduction of H^+ to H_2.[37] Conversely, [NiFe]-hydrogenases feature a bimetallic active site composed of nickel and iron ions, with the iron ion similarly coordinated by CO and CN^- ligands. The Ni site of the enzyme participates directly in catalysis, while specifically positioned surrounding iron-sulfur clusters {[3Fe,4S], [4Fe,4S]} provide pathways for electron transfer.[38,39] The high-field of CO and CN^- ligands stabilize the iron center which remains in a low-spin Fe(II) state, while the nickel center alternates between Ni(II) and Ni(III) oxidation states.[40] Hence, in both the cases, the active site is embedded in an augmented protein matrix that provides ligands for the metal ions and creates a pocket for the catalytic reaction, along with the channels for products and reactants to reach the protein surface. Generally, [NiFe]-hydrogenases are more active towards H_2 oxidation, while [FeFe]-hydrogenases excel in Hydrogen Evolution Reaction (HER). However, depending on their cellular location, hydrogenases can be modulated for either hydrogen evolution or uptake.[6,12,41] The intricate architecture of these enzymes ensures a highly efficient catalytic process, finely tuned by the enveloping protein environment. However, despite this finely tuned architecture, several challenges arise when working with hydrogenase in terms of stability. The enzyme's sensitivity to oxygen is one of the most significant hurdles, as exposure to even trace amounts can result in irreversible inactivation. Furthermore, maintaining the enzyme's structural integrity under varying thermal conditions is difficult, with high temperatures often leading to denaturation. Additionally, the enzyme's performance is highly dependent on pH, as deviations from its optimal range can disrupt the catalytic efficiency and long-term stability, making it challenging to maintain activity in diverse environments.

Hydrogenase Mimics: Challenges and Progress

Intense research is currently focused on understanding the mechanism of enzymatic function, particularly the inactivation by O_2, and on the detailed characterization of the few O_2-tolerant hydrogenases.[42,43] Therefore, designing active site analogues through a biomimetic approach is crucial for developing stable, noble-metal-free catalysts for H_2 evolution and uptake.[44,45] Over the past two decades, more than 400 new systems have been developed, focusing on mimicking the key features of the [FeFe] and [NiFe] hydrogenase in a synthetic platform.[46,47] However, many of these compounds closely resemble the natural site but employ abiological phosphine or carbene ligands, resulting in electrocatalytic proton reduction albeit at high overpotential requirements.[48–50] The synthesis of dithiolate-bridged hexacarbonyl diiron complexes, which is an analogue of the active site for [FeFe]-hydrogenases, dates back to 1929 with the work of Reihlen and colleagues.[51]

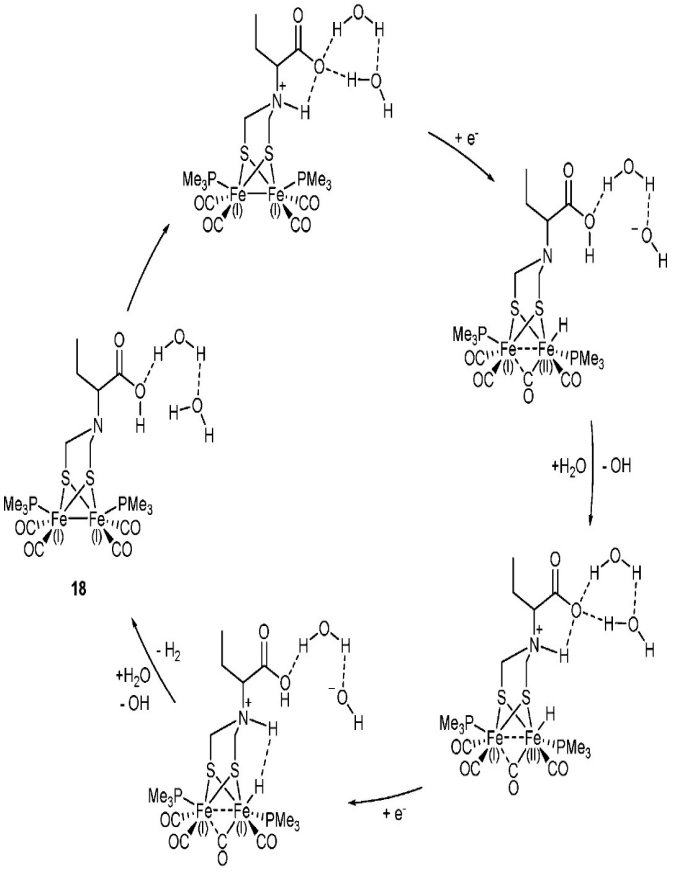


Figure 2: Proposed catalytic mechanism for the electrocatalytic reduction of H^+ in aqueous media, illustrating the mooted role of the carboxylate group as a proton transfer relay. This figure is taken from ref. [18], copyright ELSEVIER [2014]

Recent research has explored the impact of modifying the bridging group in [FeFe]-mimics, particularly by using higher group chalcogens to replace bridging dithiolates, leading to varied effects on electrocatalytic H^+ reduction.[52–56] For instance, Hou et al. synthesized an [FeFe]-mimic with a diselenolate bridge, which showcased activity towards H^+ reduction using p-toluenesulfonic acid.[57] Next, to design diiron model systems with redox properties closer to the thermodynamic potential for H^+ reduction, researchers have explored the inclusion of substituted aromatic dithiolato bridgeheads.[58] **(Fig. 2)** Another critical factor influencing the enhanced performance of enzymes is the difference between bridging and terminal binding modes of hydrides.[59,60] While most hydride models exhibit a bridging configuration and serve as active electrocatalysts for proton reduction, many studies were conducted towards the comparative catalytic studies on terminal hydride derivatives. These studies provided valuable insights into the bias of natural system toward a specific hydride mode (bridging versus terminal) and the underlying reasons for such a preference.[50] Rauchfuss and co-workers later conducted detailed investigations into the role of proton relays, suggesting that incorporating a pendant base group, like an azadithiolate bridge, would kinetically favor the formation of terminal hydride species.[49] They also proposed that this modification would lead to a lower overpotential compared to systems with bridging hydride species.[52,61–63] The exploration of electron transfer relays, which is the vital components in natural enzymes, was first attempted by Tard *et al.*, who incorporated a cubane $[Fe4S4]^-$ cluster and a $[2Fe3S]^-$ model via a sulfur ligand. Although this system functioned as an electrocatalyst for proton reduction, it had large overpotential and lacked the ability to perform the reverse process. Despite significant progress in developing [FeFe]-mimics, replicating the [4Fe–4S] cluster function has received limited attention.[64,65] In case of [NiFe] mimics, to date, only a few active synthetic catalysts have been reported, but the mononuclear $[Ni(P^R_2N^{R'}_2)_2]^{2+}$ complexes, developed by DuBois and co-workers, stand out as some of the most efficient bioinspired catalysts for hydrogen production.[18,66,67] These complexes are also the only known series capable of catalytic H_2 oxidation. The key to their high catalytic performance lies in the pendant amine groups incorporated into the diphosphine ligands of the Ni ion, which serve as crucial proton relays.

This design enables efficient H^+/H_2 interconversion at moderate overpotentials.[20] W. Shaw and co-workers have leveraged these complexes to explore the impact of enzyme-like functionalities such as amino acids, amino-ester derivatives, and peptides in the outer coordination sphere on catalytic rates **(Fig. 3A)**.[68–70] Complexes with acidic or basic side chains exhibited up to a fivefold increase in the rate of electrocatalytic H_2 evolution in water/acetonitrile mixtures compared to their unmodified counterparts **(Fig. 3B)**. Additionally, terminal propionic acid groups were employed to introduce a highly structured, sterically constrained β-hairpin 10-mer peptide into the related $[Ni(PPH_2N^{Ar})_2]^{2+}$ complex **(Fig. 3C)**.[71] However, the peptide system proved too flexible, complicating efforts to consistently study the relationship between catalytic activity and the nature of the protein-based second coordination sphere. Hence, these biomimetic systems inspired by the [FeFe] and [NiFe] catalytic cofactors found in hydrogenase active sites have struggled to match the remarkable efficiency of the natural enzymes.

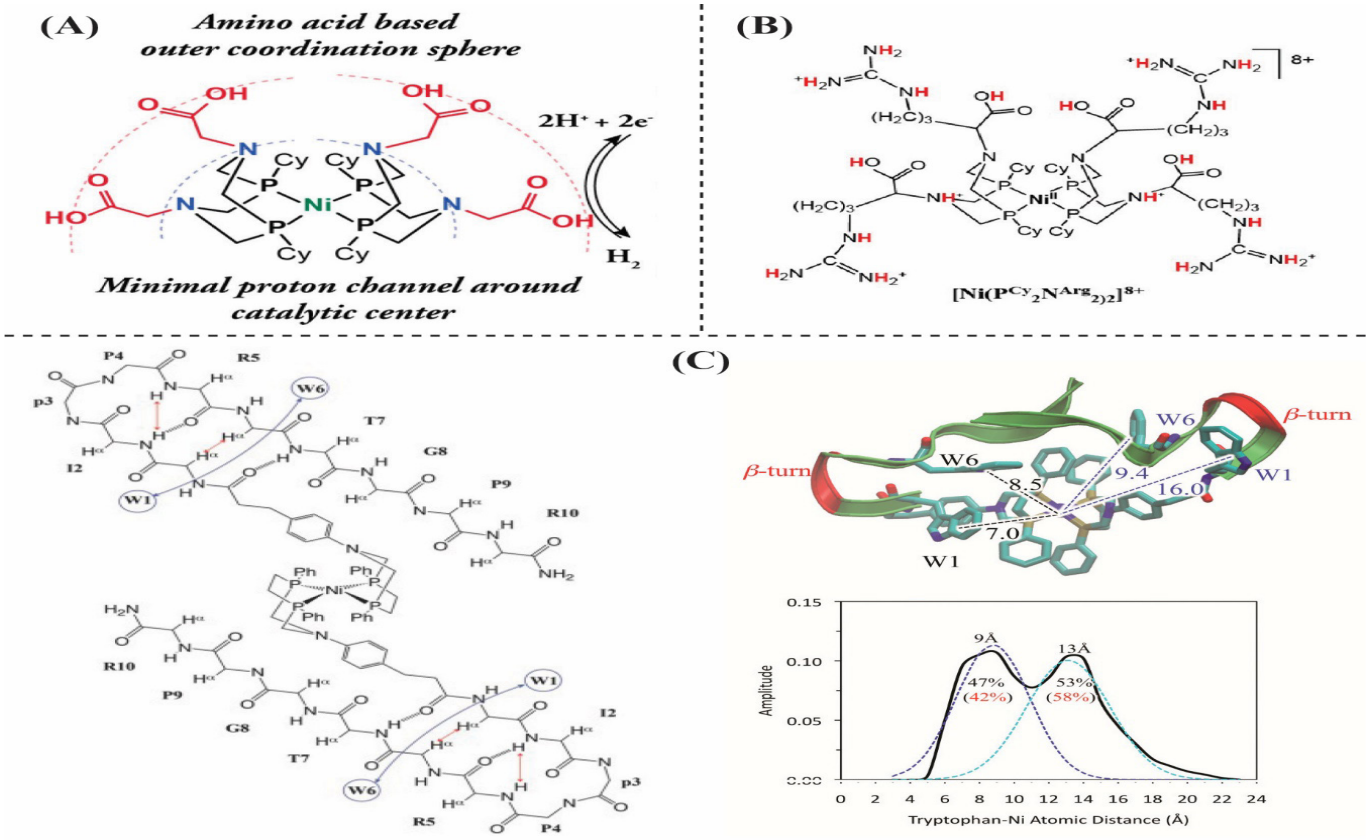


Figure 3: (A) The amine of glycine was incorporated directly into $[Ni^{II}(P^{Cy}_2N^{Gly}_2)_2]^{4+}$. The resulting complex was observed to transfer protons rapidly between the pendant amine and the COOH. This figure is taken from ref. [72], copyright ACS [2014] **(B)** $[Ni^{II}(P^{Cy}_2N^{Arg}_2)_2]^{8+}$ is an active H_2 oxidation catalyst at all pH values, with the fastest rates at low pH, conditions most relevant for fuel cells. Overpotentials also vary as

a function of pH, demonstrating a dependence on proton or electron transfer or both at high rates. This figure is taken from ref. [72], copyright ACS [2014] **(C)** $[Ni(^{PP}H_2N^{NNA-hairpin})_2]^{2+}$ complex is an active H_2 production catalyst. This figure is taken from ref. [69], copyright ACS [2014]

This distinct gap in reactivity between the enzymatic systems and these synthetic small molecule models can be attributed to two key factors: (i) the need for the synthetic models to operate in non-aqueous solvents, and (ii) the absence of a protein environment, which plays a crucial role in modulating catalytic activity through outer coordination sphere interactions.

Experimental aspects of testing Enzymes for hydrogen production:

To evaluate enzyme-catalyzed hydrogen production, cyclic voltammetry (CV), bulk electrochemistry, and gas chromatography (GC) are commonly employed. Initially, CV studies are performed in a typical three-electrode setup consisting of a working electrode, reference electrode, and counter electrode. The enzyme is immobilized on the working electrode surface within an electrochemical cell, and the potential is swept over a defined range to monitor its redox and catalytic behavior. This technique provides valuable insights into the enzyme's electrochemical activity, onset potential, overpotential, and overall efficiency in hydrogen production. The catalytic current observed during CV directly correlates with the enzyme's ability to produce hydrogen under specific conditions. To further evaluate hydrogen production, bulk electrochemistry is performed after CV. In this step, the enzyme-coated electrode is held at a constant potential for an extended period, enabling sustained hydrogen generation. Once hydrogen is produced electrochemically, GC is used for quantification. The headspace gas from the electrochemical cell is sampled and injected into the GC system, where the hydrogen is separated and detected, providing precise measurements. Together, these techniques offer a comprehensive analysis of the enzyme's hydrogen production capability.

Transition to Proteins and Peptides: Incorporating Artificial Molecular Catalysts into Proteins

The exceptional performance of metalloenzymes is largely due to the precise control over reactivity, which is achieved through a complex interaction between the metal at the active site, the surrounding metal-coordinating atoms or the outer coordination sphere (OCS) and the dynamic protein scaffold that both encapsulates and extends beyond the active site. Bioinorganic research has been keenly focused on unraveling the structure-function relationships essential for this controlled reactivity.[73–77] Recent studies have highlighted the protein scaffold as a highly versatile element, significantly influencing metalloenzyme chemistry.[78,79] Although the full extent of the protein scaffold's impact is not yet fully understood, it is believed to directly affect catalytic activity in several ways: (1) fostering favorable interactions between the scaffold and the metal or cofactor, (2) strategically positioning residues and excluding solvent to regulate the electrostatic environment, (3) embedding active sites within specific pockets to facilitate the entry of reactants while limiting solvent or inhibitor access, and (4) enabling or restricting conformational changes throughout the catalytic cycle.[80] Various synthetic strategies have been employed to replicate the influence of enzymatic scaffolds on catalytic activity within synthetic systems. One prominent approach is the reengineering of metal-binding proteins. Early work by Lombardi and Lu's groups

showcased successful examples of such protein reengineering.[77] Building on this, Shafaat and colleagues later redesigned rubredoxin, substituting Ni for Fe, thereby creating a simplified structural and functional model of [NiFe]-hydrogenase.[81] This engineered Ni-complex demonstrated significant electrocatalytic activity for H_2 production, unlike the Fe-complex, which did not exhibit such activity. The active site surrounding Ni effectively mimics the geometry of [NiFe]-hydrogenase. Further investigations utilizing molecular dynamics simulations, resonance Raman spectroscopy, and quantitative electrochemical analysis identified a plausible reaction mechanism, where thiol inversion followed by hydride formation constitutes the rate-limiting step. Modifying the second coordination sphere with two independent valine-to-histidine variants revealed distinct roles for this outer coordination sphere. The V8H construct exhibited nearly a four-fold increase in catalytic activity compared to the wild type (WT), whereas the V34H variant maintained similar activity levels.

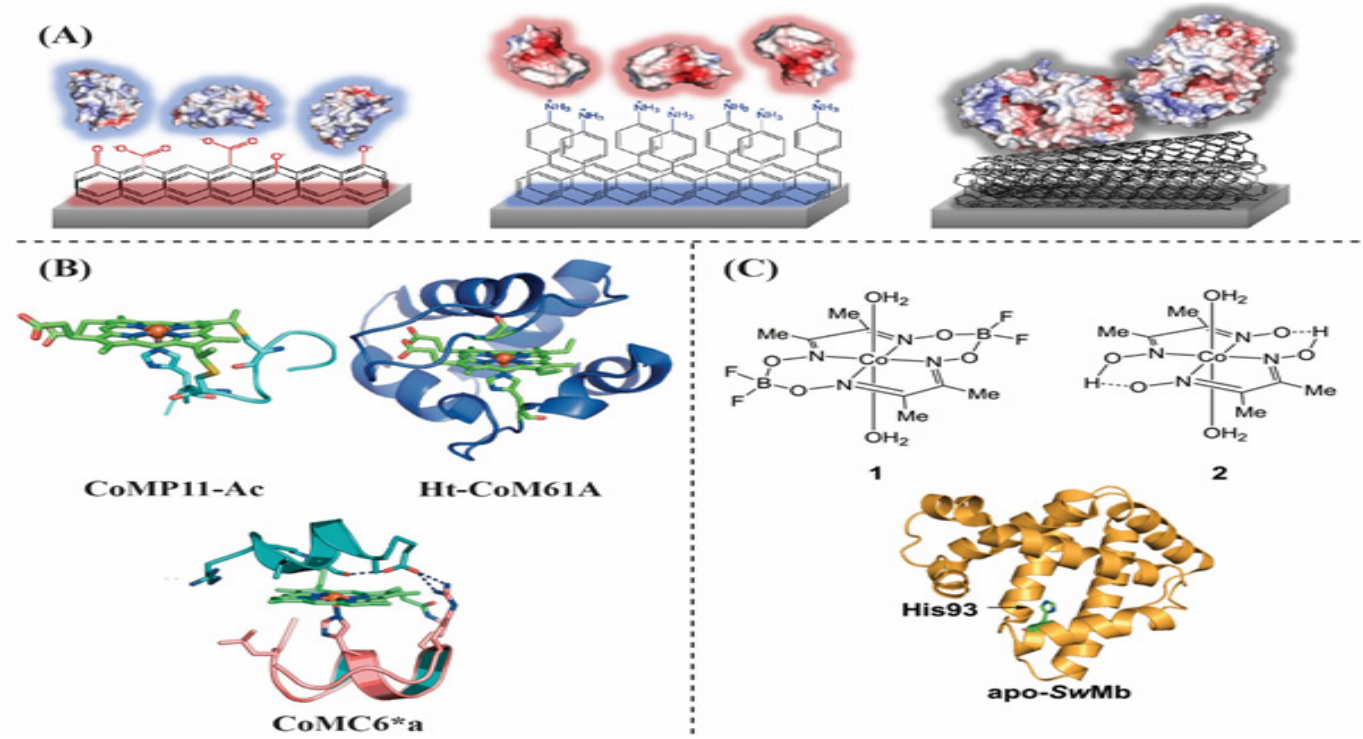


Figure 4: (A) Surface of unmodified graphitic electrodes is typically negatively charged, favoring electrostatic adsorption of positively charged proteins and molecules [left]. Aniline-functionalized surfaces are positively charged, favoring electrostatic adsorption of positively charged proteins and molecules [centre]. Nanotube-coated surfaces are hydrophobic, favoring adsorption of large, hydrophobic, or zwitterionic proteins and molecules [right]. This figure is taken from ref. [82], copyright ACS [2020] **(B)** Figures of cytochrome c-derived heme-peptide complex, microperoxidase-11 **CoMP11-Ac (PDB ID: 1CRC)**, a cobalt-substituted mutant of *Hydrogenobacter thermophilus* cytochrome c552 **Ht-CoM61A (PDB ID: 1AYG)**, a synthetic cobalt-heme mini-protein **CoMC6*a** (Model). This figure is taken from ref. [83], copyright ACS [2019] **(C)** Structures of the model of **apo-SwMb** (sperm-whale myoglobin) showing H93 of the binding pocket (PDB ID: IUFJ) This figure is taken from ref. [83], copyright ACS [2014].

Notably, the V8H mutant showed turnover frequencies (TOFs) that were independent of pH, in contrast to the V34H mutant, which was pH-dependent. These findings underscore the outer coordination sphere's sensitivity to subtle environmental changes.[84] Another crucial factor in improving the efficiency of these systems is achieving rapid interfacial electron transfer rates between the enzyme and electrode, which enables precise electrochemical control of redox-active enzymes immobilized on the electrode surface. Pioneering electrochemical studies by Armstrong and co-workers demonstrated that even minimal amounts of [NiFe] hydrogenase adsorbed on pyrolytic graphite electrodes could produce remarkably high catalytic currents, allowing for the measurement of catalytic and affinity constants.[29] More recently, Shafaat and co-workers have elaborated on various methodologies for the covalent attachment of nickel-substituted rubredoxin (NiRd) to unmodified graphite electrodes through amide bond formation **(Fig. 4A)**.[82] The resulting electrodes exhibited high activity for H_2 evolution over several weeks. This simple and effective approach has the potential to be widely applied to various redox-active proteins and enzymes, enhancing the stability of these systems over extended periods. However, challenges remain, such as reduced protein dynamics and limited oxygen tolerance in some cases, which continue to hinder their practical applications. To tackle this challenge, various cobalt complexes and their bio-hybrids have demonstrated promising potential for enhanced hydrogen production. Noteworthy contributions in this field have been made by Bren and team. One of the earliest and simplest catalysts developed is CoMP11-Ac **(Fig. 4B)**, a cobalt-porphyrin-based catalyst that operates in water and boasts a respectable turnover number (TON) of 25,000. This catalyst is synthesized by substituting cobalt for iron in the cytochrome c-derived heme-peptide complex, microperoxidase-11 (MP11), followed by acetylation of the free amines. CoMP11-Ac features cobalt protoporphyrin IX covalently linked to an 11-amino acid chain through a Cys-Xaa-Xaa-Cys-His motif, providing an axial histidine ligand on the porphyrin's proximal side. Unlike many porphyrin-based catalysts, CoMP11-Ac is functional in water. However, its short lifespan spurred the development of other biocatalysts with a similar cobalt porphyrin active site, but with polypeptides on both sides of the porphyrin. One such catalyst is Ht-CoM61A[85] **(Fig. 4B)**, a cobalt-substituted mutant of *Hydrogenobacter thermophilus* cytochrome c552, which is more robust, lasting approximately 5 hours and achieving a tenfold increase in TON to 270,000. Another notable cobalt-porphyrin-based catalyst is the synthetic cobalt-heme mini-protein CoMC6*a[86] **(Fig. 4B)**, which not only lowers overpotential but also enhances catalyst longevity, with a TON of 230,000. Another strategy for designing catalysts involves enhancing proton availability and delivery to the active site. One example of this approach is the addition of a bioinspired outer-coordination sphere to the widely studied Cobaloxime, which is known for its excellent oxygen tolerance and water solubility. Artero and co-workers developed two cobaloxime derivatives featuring difluoroboronyl (1) and proton-bridged (2) glyoxime ligands, which were incorporated into sperm-whale myoglobin, resulting in SwMb·1 and SwMb·2, respectively **(Fig. 4C)**.[87] These hybrids demonstrated activity in photocatalytic hydrogen production. Although their turnover numbers (TON) were modest (~5), this work successfully introduced synthetic catalysts into a protein scaffold, creating a water-compatible system with H_2-evolution activity. Later, Lisa M.

Utschig and her team incorporated cobaloxime into ferredoxin, which improved its TON (~85) while maintaining its activity only towards photocatalytic H_2 production capability.

Over the past five years, our group has been dedicated to enhancing the activity of cobaloximes through modifications with amino acids, neurotransmitters, vitamin B6, drug molecules, and nucleic bases. We observed that each modification, featuring an improved outer coordination sphere with strategically positioned basic functionalities, led to a significant increase in electrocatalytic hydrogen production. To further explore the untapped potential of this catalyst, we are currently exploring the possibility of incorporating the cobaloxime core into a robust protein scaffold (like azurin) to create a synthetic metalloprotein. Here we aim to harness the unique interplay between the transition metal active site and the protein-based OCS. Azurin (Az) exemplifies a sophisticated synergy between the Cu-based active site and the adaptable protein-based outer coordination sphere (OCS), while it remains resilient to mutation in the protein scaffold. Hence, Az provides a perfect tuneable platform, providing a well-defined secondary coordination sphere to host variable metal cofactors and investigate the resulting catalytic efficiency and selectivity. Additionally, this durable constructs can be integrated into operational electrolyzers for generating hydrogen from neutral water, further underscoring its applicability in diverse real-world scenarios.

Current Challenges and Future Perspectives

While the field of hydrogen production using enzymes, proteins, and peptides has made remarkable strides, several significant challenges remain that must be addressed to realize the full potential of these bio-inspired systems in practical applications. While hydrogenase enzymes are highly efficient in their natural contexts, replicating this efficiency in synthetic or biohybrid systems remains difficult. Synthetic mimics often struggle to achieve the same turnover numbers and overpotentials as their natural counterparts. Additionally, integrating enzymes or synthetic mimics into practical devices like electrolyzers or fuel cells poses its own set of challenges. The delicate nature of these catalysts requires careful control of the operational environment to maintain their activity and stability. Moreover, the need for efficient electron transfer between the catalyst and the electrode materials adds another layer of complexity. Achieving the optimal interface between the biological or biohybrid catalyst and the inorganic components of a device is crucial for maximizing performance but remains a significant engineering challenge. Hence, an ideal enzyme should be robust, exhibit thermal stability and resistance to harsh conditions, such as varying pH levels and high temperatures, which are common in industrial processes.

Advances in protein engineering and synthetic biology offer exciting possibilities for enhancing the oxygen tolerance and stability of hydrogenase enzymes. By introducing mutations that protect the active site from oxygen damage or by encapsulating the enzyme in protective matrices, it may be possible to extend the utility of these catalysts to a wider range of environments. Also, the integration of synthetic catalysts into protein frameworks represents a promising strategy to combine the best attributes of both natural and synthetic systems. These bio-hybrid systems could leverage the structural complexity and specificity of proteins while benefiting from the robustness and scalability of synthetic catalysts. Furthermore, the development of multi-component catalytic systems, where different components work

synergistically to enhance overall performance, could lead to breakthroughs in both efficiency and stability. Moreover, scaling up these systems from laboratory experiments to industrial-scale applications will require close collaboration between researchers in catalysis, materials science, and chemical engineering. Demonstrating the viability of these systems in real-world applications, such as in electrolyzers that operate on seawater or other challenging environments, will be a key step toward their broader adoption.

Conclusion

The quest to harness the efficiency of hydrogenase enzymes for practical hydrogen production has driven significant advancements in both biological and synthetic catalysis. Despite their unmatched catalytic prowess, the challenges of oxygen sensitivity, stability, and scalability have limited the direct application of hydrogenases in industrial settings. This mini-review has highlighted the journey from natural enzymes to innovative biohybrid and synthetic systems that seek to replicate and surpass the performance of hydrogenase catalysts. Efforts to engineer stable catalysts, exploring alternative metal centers, and developing biohybrid systems have shown promise in addressing these challenges. Moreover, the integration of these catalysts into practical devices, such as electrolyzers, operational in seawater are the tools to guide catalyst design as critical steps forward. As the field progresses, the development of robust, efficient, and scalable hydrogen production technologies will be essential for the transition to a sustainable hydrogen economy. The future of hydrogen production lies in the successful combination of nature's design principles with human innovation, leading to systems that not only match but exceed the capabilities of their natural counterparts. Continued research and interdisciplinary collaboration will be key to overcoming the remaining obstacles and realizing the full potential of these bio-inspired approaches in the energy landscape of the future.

Conflicts of interest

The authors declare no conflicts of interest.

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