# Investigating the Key Bridging Ligand in the Active Site of [FeFe] Hydrogenase Enzymes Towards the Development of Artificial Metalloenzymes for Hydrogen Conversion


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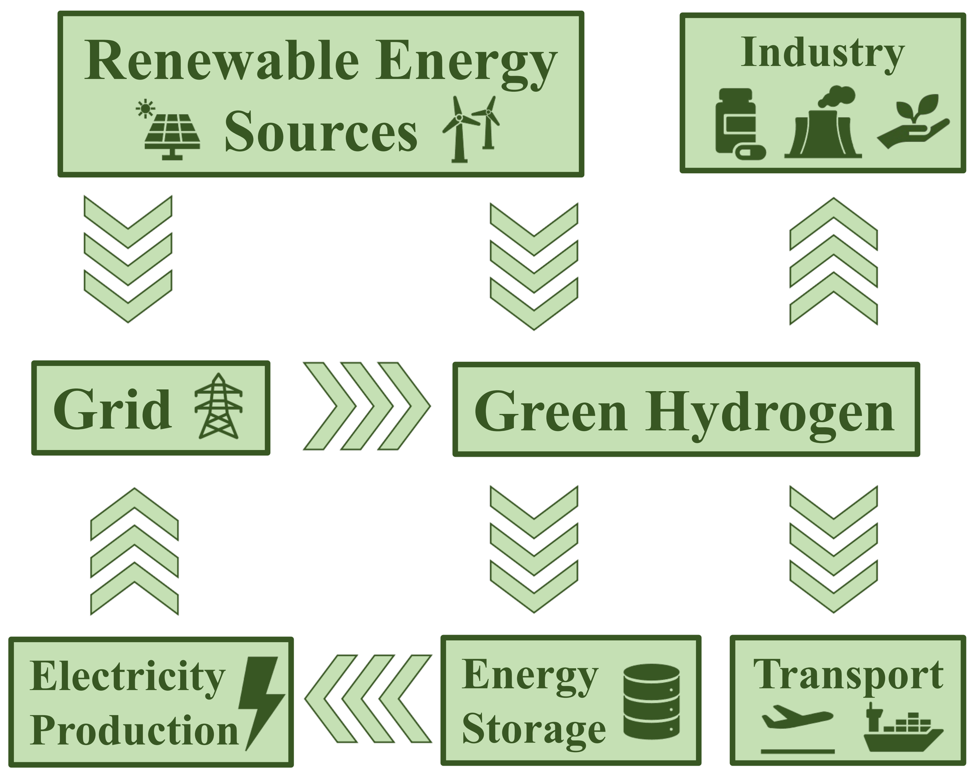
## 1. The Potential of Hydrogen in a Sustainable Future

### How do we supply reliable, affordable, and renewable energy to all?

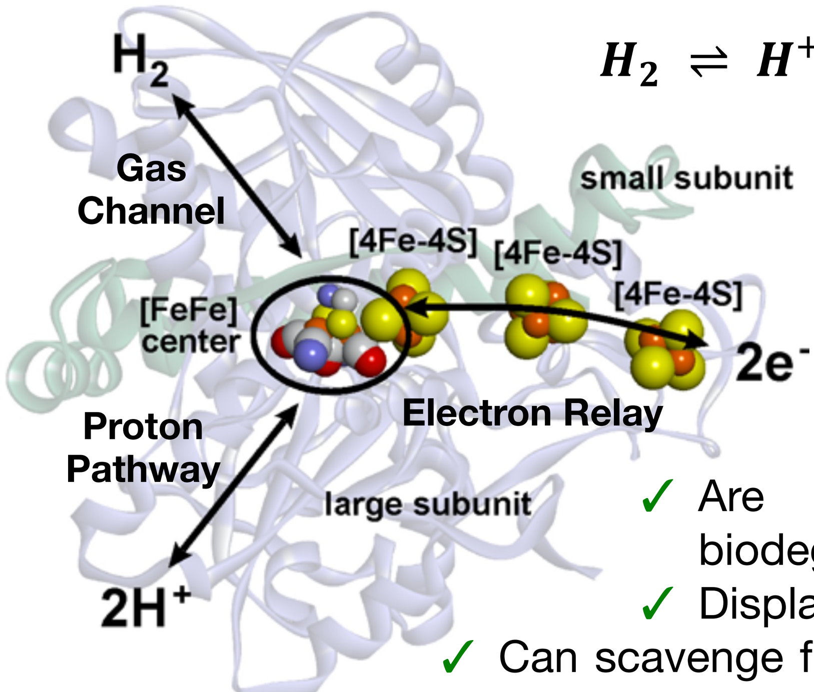
- ➪ As renewable energy is an intermittent energy source it requires an energy vector for storage and transportation.[1-4]
- ➪ Hydrogen is the simplest energy vector to manufacture, storing more energy per unit volume and producing zero emissions.[5]
- ➪ Renewables can provide energy to split water into hydrogen and oxygen in an electrolyser, generating chemical energy through strong H-H bonds.[6]
- ➪ Hydrogen can be oxidised to water in a fuel cell to generate electricity.
- ➪ There are currently no sustainable catalysts for global hydrogen production/oxidation for use in electrolysers and fuel cells.[7,8]




## 2. Hydrogenase Enzymes

Nature's hydrogen economy formed billions of years ago with the evolution of **hydrogenase enzymes**.[8,9]

$$H_2 \rightleftharpoons H^+ + H^- \rightleftharpoons 2H^+ + 2e^-$$




They are **advantageous** to use in fuel cells and electrolysers as they:[3,8,10-13]

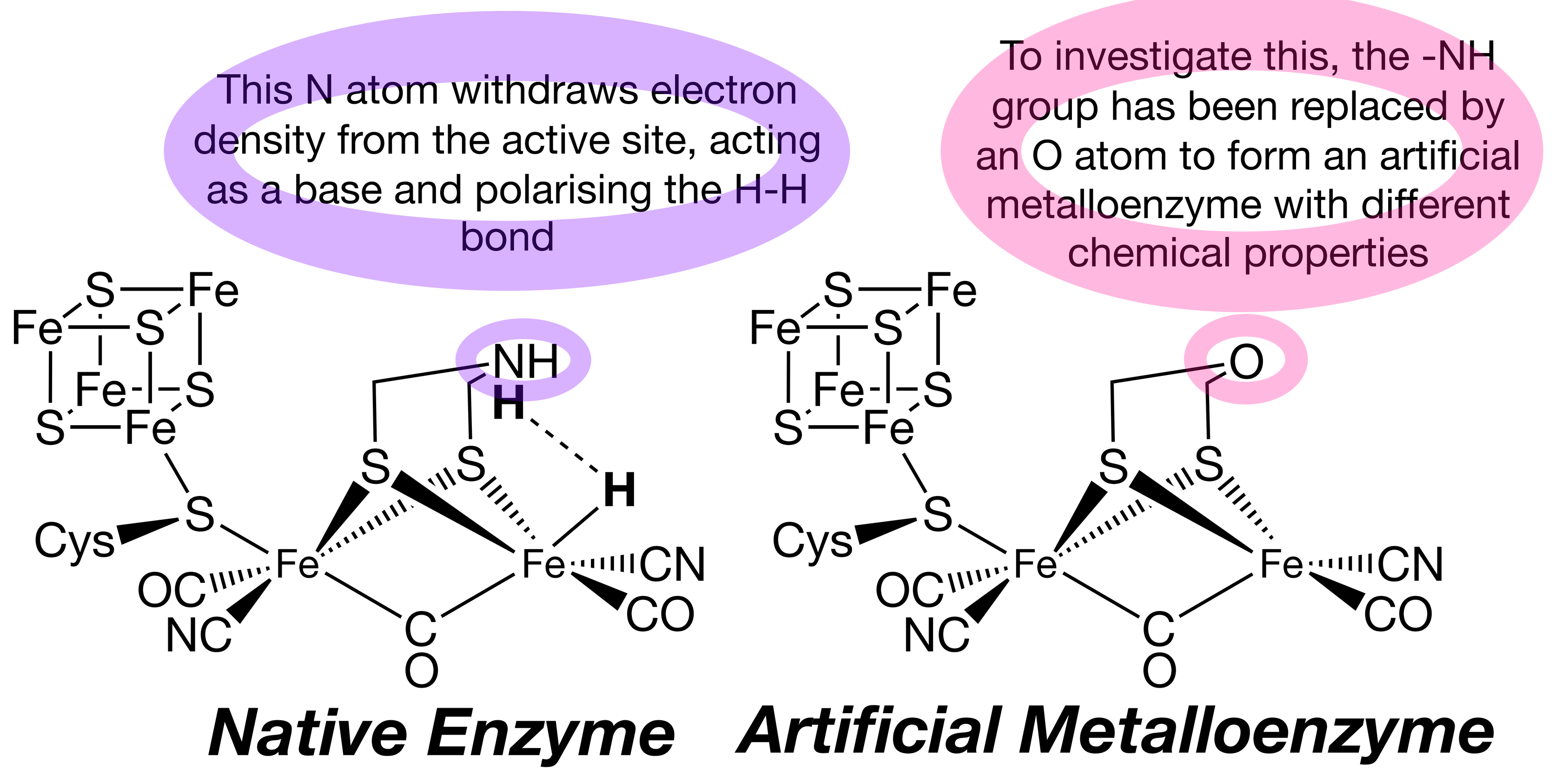
- ✓ Exhibit very high catalytic rates under ambient conditions
- ✓ Are infinitely renewable and biodegradable
- ✓ Display excellent specificity
- ✓ Can scavenge fuel and oxidants in very low concentrations
- ✓ Are very efficient and fast catalysts when adsorbed on electrode surfaces

However, their industrial applications are **limited** because:[3,10,12-15]

- ✗ They are often unstable under harsh conditions
- ✗ Their isolation and purification are arduous techniques, which are difficult to scale up to commercial levels
- ✗ Exposure to oxygen causes mainly irreversible deactivation

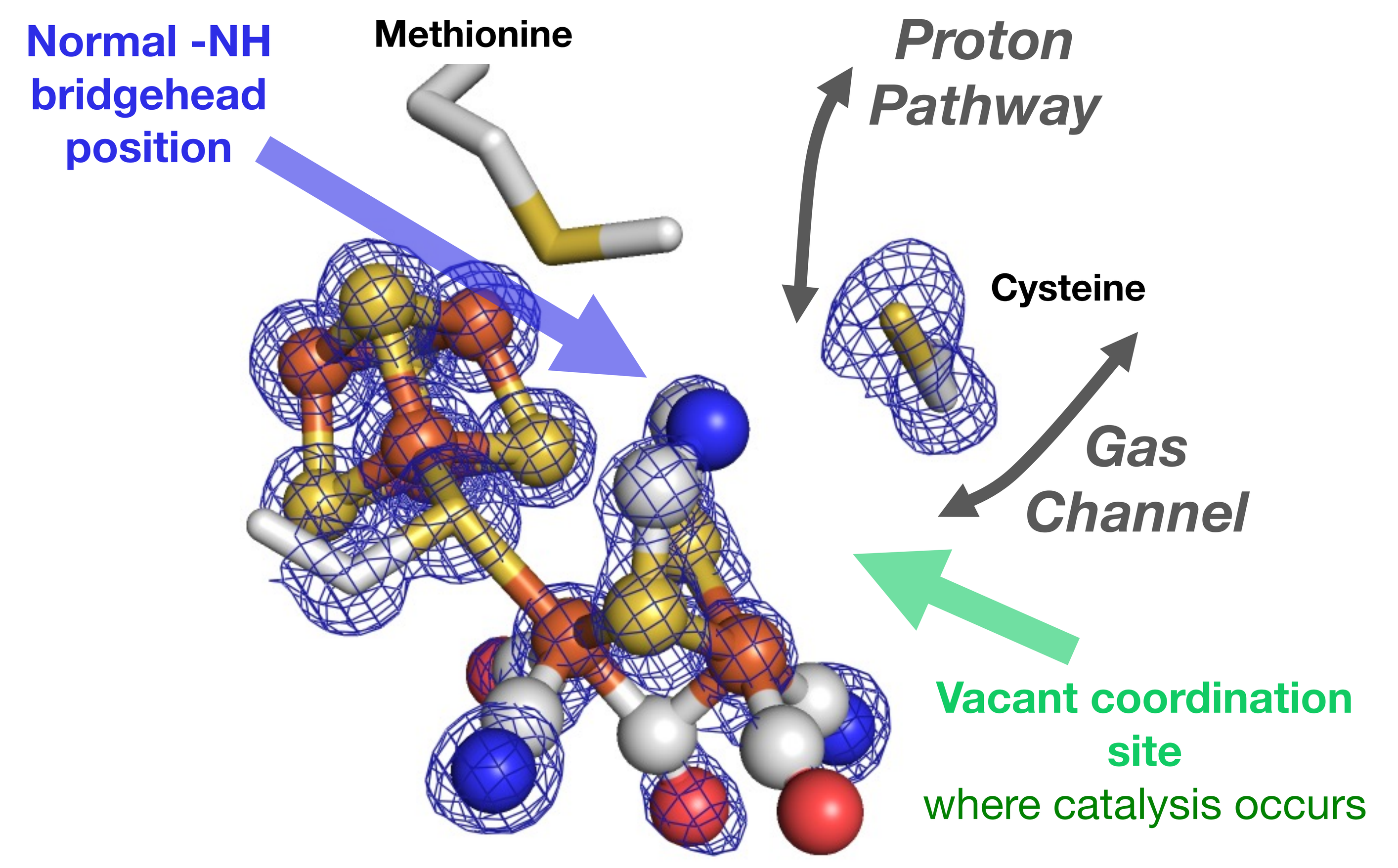
## 3. [FeFe] Hydrogenases

The [FeFe] hydrogenase is the **fastest-known biological catalyst** for hydrogen production.[14]

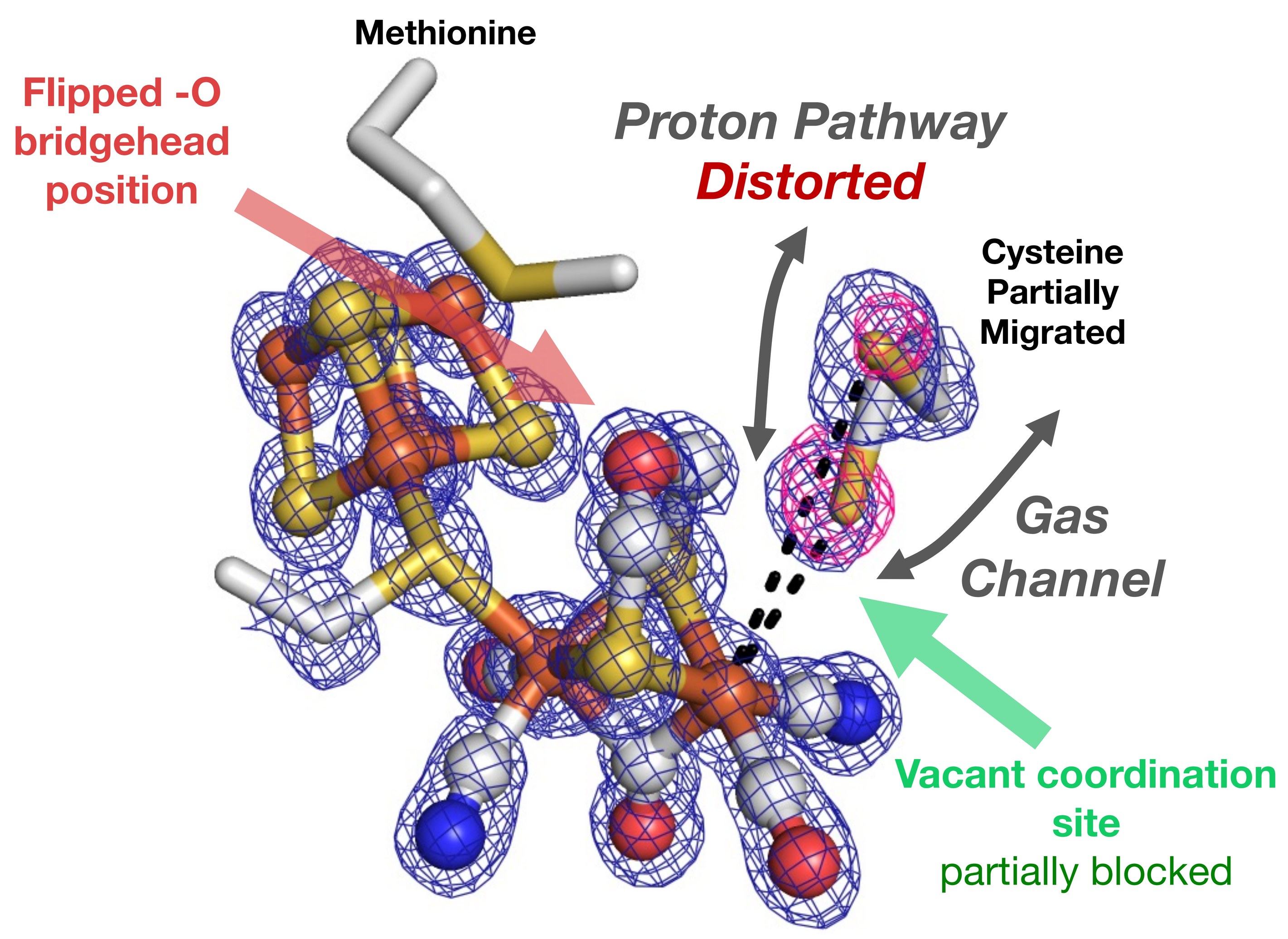



Further understanding the role of this -NH group and its interactions with $H_2$ and the enzyme's protein scaffold is crucial for developing commercial synthetic mimics or bioinspired catalysts.

## 4. Active-Site Structure: X-Ray Crystallography



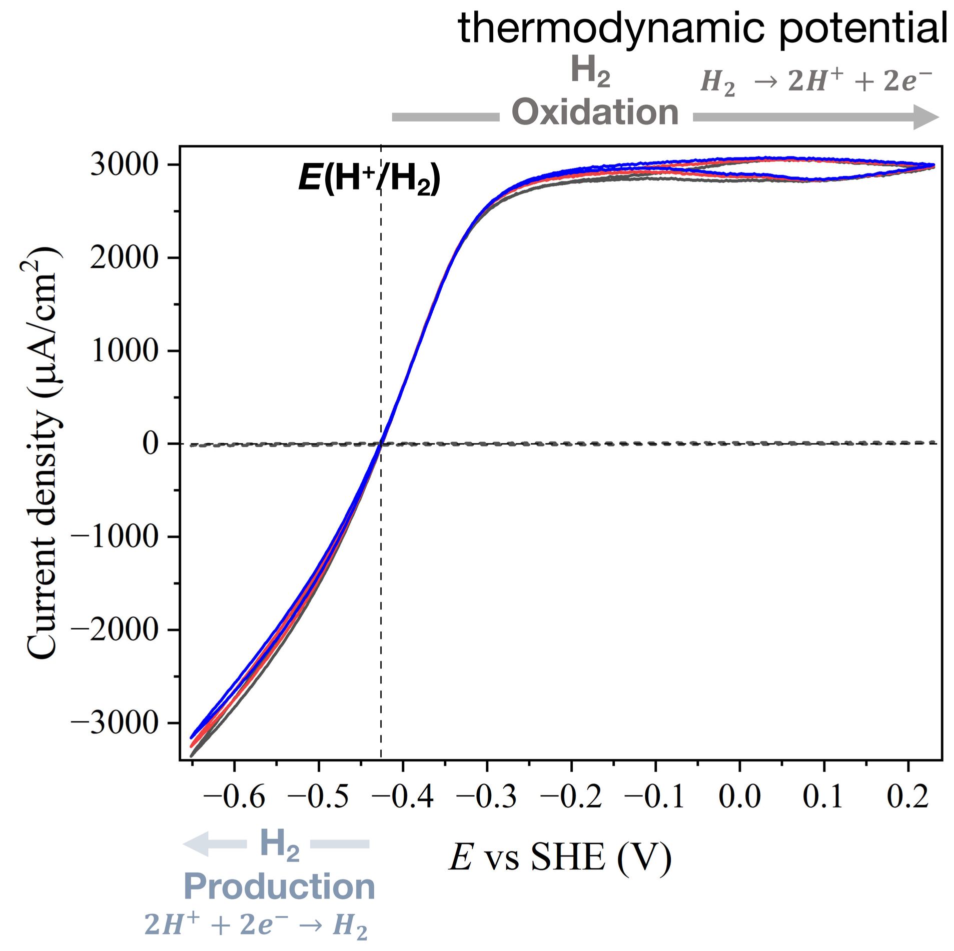

**Native Enzyme** (0.99 Å resolution)

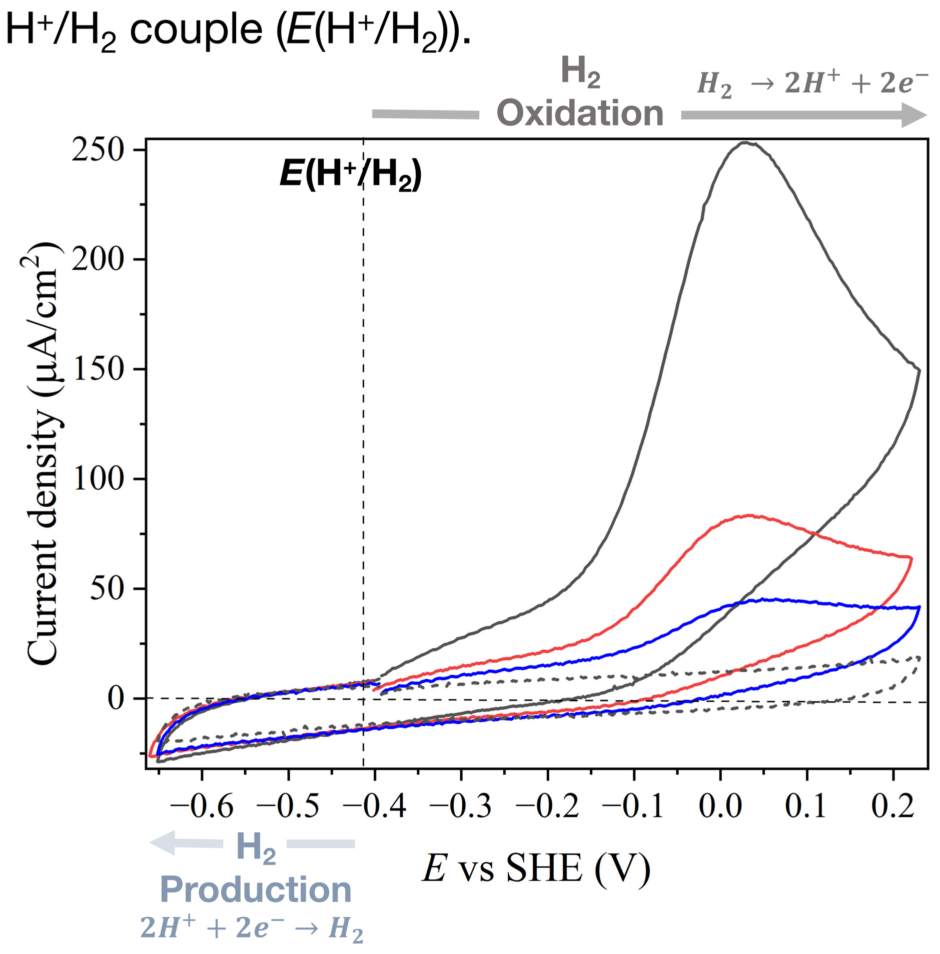



**Artificial Metalloenzyme** (0.96 Å resolution)

## 5. Catalytic Activity: Electrochemistry

$H_2$ oxidation/production depends on whether the applied potential is positive or negative relative to the thermodynamic potential of the $H^+/H_2$ couple ($E(H^+/H_2)$).




### Native Enzyme

- Exhibits very high catalytic current densities for both $H_2$ oxidation and production.
- Reversible high-potential inactivation is observed at high positive potentials.

### Artificial Metalloenzyme

- Exhibits smaller current densities and only in the $H_2$ oxidation direction; no $H_2$ production observed.
- Irreversible high-potential inactivation is observed at high positive potentials.

## 6. Conclusions

- ➪ The flipped bridgehead of the artificial metalloenzyme is likely enabled by the smaller size of O and the weakened interactions with the protein scaffold's amino acid residues.
- ➪ Highlights the **importance that the amino acids have in maintaining the correct bridgehead position geometry for optimal catalysis** by interacting with the active site.
- ➪ The partial migration of the cysteine residue into the vacant coordination site in the artificial metalloenzyme allows for **greater protection of the active site towards $O_2$ degradation while maintaining catalytic activity**.
- ➪ This provides promise for developing a system that utilises a 'flipped bridgehead' mechanism for enhanced $O_2$ stability in artificial metalloenzymes or synthetic catalysts and **overcome one of the main issues of the native enzymes that greatly hinders their commercial applicability**.

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