UNISENSE & SCIENTIFIC

Hydrogen production by a fully de novo enzyme

Quantifying the Hydrogen Evolution Reaction using H_2 Microsensors

The application note is based on the research and article by:

Berglund et al.

The application note is written by:

Petersen et al., Unisense

Introduction

Hydrogen utilization is a promising alternative to fossil fuels where molecular, metal-based catalysts represent a sustainable opportunity. However, many molecular catalysts require rare metals or organic solvents, reducing their sustainable potential.

Cobaloxime is a molecular catalyst containing the globally available metal, cobalt, but its low solubility and stability has hindered its broader application. In a study from 2024, Berglund et al. sought to address this challenge by designing a de novo artificial enzyme for hydrogen production.

"The Unisense H_2 Microsensors have been massively useful in my research. Being able to continuously monitor the hydrogen evolution directly in solution gives me immediate insight into the behaviour of my catalysts.

The sensors and the software are easy to use, and when I have had any questions or issues arise they have always been quickly resolved by their staff."

PhD candidate Sigrid Berglund,
Department of Chemistry, Uppsala University

The researchers engineered a 65-amino acid protein consisting of three alpha-helices and covalently linked cobaloxime to a cysteine residue. This would effectively solubilize cobaloxime, but also partially bury it within the protein scaffold, potentially reducing the efficacy of hydrogen production from the artificial enzyme compared with free cobaloxime.

Laboratory Setup

The researchers quantified the Hydrogen Evolution Reaction (HER) of the artificial enzyme and compared it to free cobaloxime. Both catalysts were placed in sealed vials with septum closures to prevent gas exchange with the atmosphere, and hydrogen production was initiated either photocatalytically or chemically.

For the photocatalytic HER, the artificial enzyme and free cobaloxime were illuminated in the presence of $[Ru(bpy)_3]^{2+}$ and ascorbic acid as an electron donor.

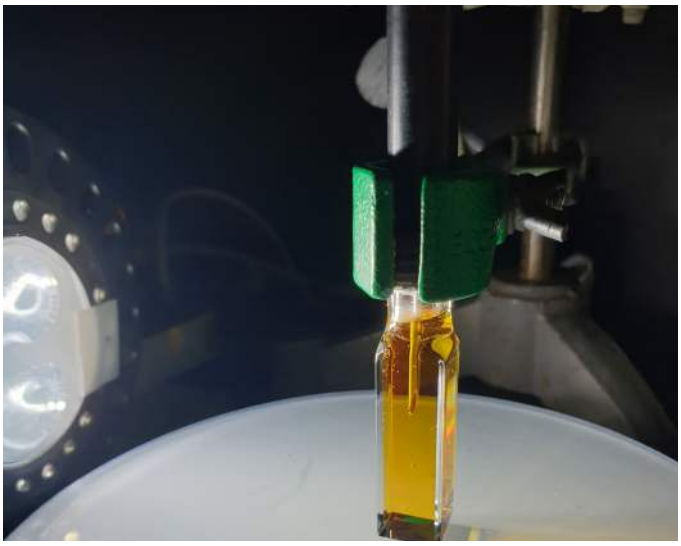

Figure 1: The Unisense H_2-NPLR piercing needle sensor inserted into the vials with the artificial enzyme.

A Unisense H_2 Microsensor in a piercing needle (H_2-NPLR) was inserted through the septum and submerged in the solution (Figure 1). Once HER was triggered, the microsensor continuously monitored hydrogen levels in real time.

Results and conclusion

Under photocatalytic conditions, the artificial enzyme exhibited a rapid initial increase in hydrogen production, reaching a peak concentration of approximately 49 µM per µM catalyst (Figure 2). Free cobaloxime showed a similar trend but achieved a higher maximum hydrogen concentration of 62 µM per µM catalyst.

Consequently, the hydrogen production of the artificial enzyme corresponds to 80% of the hydrogen production of free cobaloxime, indicating a slightly reduced catalytic efficiency when cobaloxime was embedded in the protein scaffold. The researchers thereby demonstrated the potential of using artificial enzymes for hydrogen production.

The Unisense H_2 Microsensor enabled continuous, real-time measurements of dissolved hydrogen throughout the illumination period. Its piercing needle design allowed insertion into the sealed vials while avoiding gas exchange with the atmosphere.

You can read more in the article by Berglund et al. "Hydrogen production by a fully de novo enzyme", Dalton Trans., 2024, 53,12905

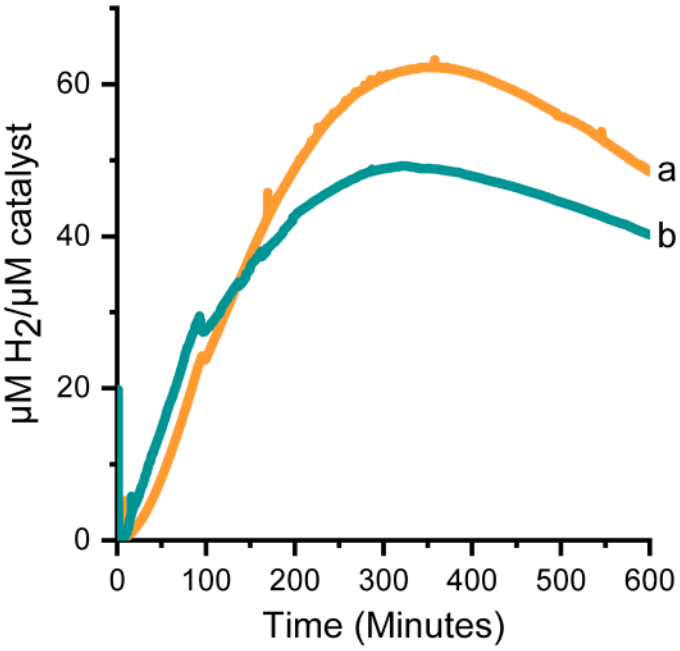

Figure 2: The Hydrogen Evolution Reaction of free cobaloxime (a) and the artificial enzyme (b) as measured by the Unisense H_2-NPLR sensor.

Suggested products

- H_2-NP
- OX-NP
- Temp UniAmp
- Single or MultiChannel UniAmp
- uSense Log

Related publications

Hou, Heting, et al. "Enhanced electrocatalytic hydrogen evolution with bimetallic Ru/Pt nanoparticles supported on nitrogen-doped reduced graphene oxide." Inorganic Chemistry Frontiers (2025).

Appel, Jens, et al. "Cyanobacterial in vivo solar hydrogen production using a photosystem I–hydrogenase (PsaD-HoxYH) fusion complex." Nature Energy 5.6 (2020): 458-467.

Wang, Degao, et al. "A molecular tandem cell for efficient solar water splitting." Proceedings of the National Academy of Sciences 117.24 (2020): 13256-13260.

Sick, Torben, et al. "Oriented films of conjugated 2D covalent organic frameworks as photocathodes for water splitting." Journal of the American Chemical Society 140.6 (2018): 2085-2092.

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