# Design, production and evaluation of a *de novo* enzyme for hydrogen evolution

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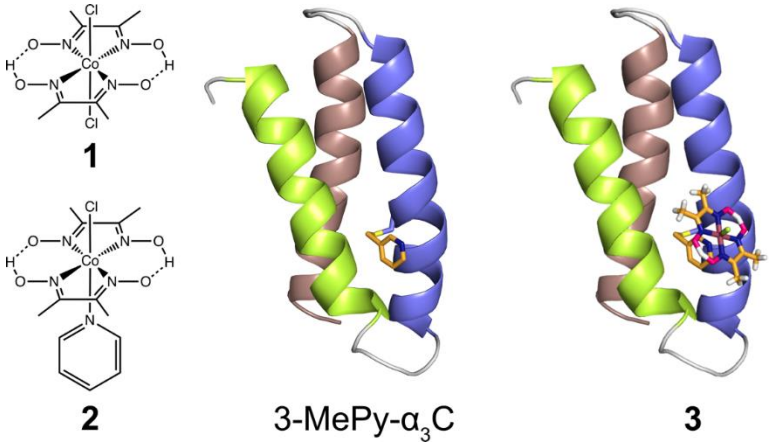
To explore new avenues in the field of sustainable production of the energy storage solutions of the future, we have designed, produced and tested a fully *de novo* enzyme for hydrogen evolution (complex **3**, see figure) (1). The enzyme is based on a coiled coil triple helix protein scaffold $\alpha_3C$ (2), functionalised with a cobaloxime (complex **2**, see figure) (3). Cobaloxime has been studied as a hydrogen evolving catalyst since the 1980s, but is unstable in aqueous soliton (4). By incorporating it into a stable protein such as $\alpha_3C$, we aim to showcase the use of man-made components to create a fully *de novo* enzyme. The use of protein scaffolds may shield the cobaloxime from the bulk solution, ensuring improved stability when used in aqueous solution. This would make it more viable for potential upscaled systems, where organic solvent can be excluded. We have conceived of a three-step functionalisation method where an unnatural amino acid is created *in vitro* (3-MePy-$\alpha_3C$, see figure) to which the cobaloxime is coordinated. The functionality of complex **3** was tested by both a photochemical approach and a chemical reduction approach, and evolved hydrogen was quantified by Clark-type electrode and gas chromatography (1), and compared to the results of that of complex **2**.

To further expand our understanding of the *de novo* enzyme, we are currently investigating catalysis at different potentials using chronoamperometry and chemical reduction assay, with Clark-type electrode hydrogen sensing. To gain insight into the mechanisms taking place at these different potentials, we are using EPR-spectroscopy to probe the accumulation of Co(II)-species.




1: S. Berglund et al., *Dalton Trans.*, 2024, 53, 12905
2: C. Tommos et al., *Biochem.*, 1999, 38:29, 9495
3: P. Connolly and J. H. Espenson, *Inorg. Chem.*, 1986, 25:16, 2684
4: P. Du et al., *Inorg. Chem.*, 2009, 48:11, 4952