How accessory iron-sulfur clusters influence catalytic bias, O_2 tolerance and overpotential in [FeFe] hydrogenases

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[FeFe] hydrogenases interconvert H_2 with H^+ and e^- at high rates under ambient conditions and hold promise for H_2-producing devices and fuel cells. Their active site, the H-cluster, is composed of a [4Fe-4S] cluster ($[4Fe\text{-}4S]_H$) covalently linked to a diiron subsite ($[2Fe]_H$).[1] The M3 type [FeFe] hydrogenases are particularly interesting because of their catalytic bias toward H_2 production and their lower sensitivity to O_2 compared to other [FeFe] hydrogenases. As well as the H-cluster domain, containing the active site, they possess an F-domain with electron relay iron-sulfur (FeS) clusters (F-clusters). The reason for the characteristic properties of the M3 type [FeFe] hydrogenases is not clearly understood. In this study, we produce the M3 type [FeFe] hydrogenase from *Clostridium acetobutilicum* (*Ca*HydA) in *E. coli* as an apo-enzyme (lacking $[2Fe]_H$) and reconstitute $[2Fe]_H$ *in vitro* with synthetic cofactors. This allowed systematic truncation of the FeS clusters containing F-domain to investigate their influence on catalysis and the redox behavior of the H-cluster. Successive removal of the different FeS clusters resulted in variants with distinct spectroscopic properties, activities and O_2 sensitivity but, surprisingly, the catalytic bias and overpotential for catalysis was unaffected. Our results suggest that the entry point for electrons is not the determining factor of the catalytic bias in this enzyme. In a broader context, our work illustrates how [FeFe] hydrogenases can be engineered to produce active variants with specific properties.

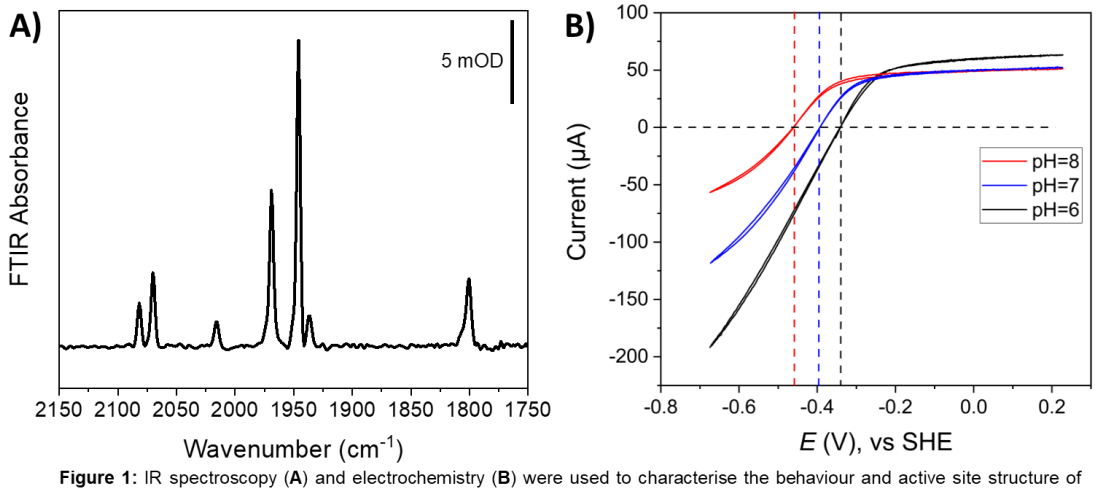

Figure 1: IR spectroscopy (**A**) and electrochemistry (**B**) were used to characterise the behaviour and active site structure of the truncated variants.

[1] W. Lubitz, H. Ogata, O. Rüdiger, and Ed. Reijerse *Chem. Rev*. **2014**, *114*, 4081–4148