# Electrochemical Investigations of the Interconversion between Catalytic and Inhibited States of the [FeFe]-hydrogenase from *Desulfovibrio desulfuricans*

Alison Parkin[1], Juan Fontecilla-Camps[2], Fraser A. Armstrong[1]

*[1]Inorganic Chemistry Laboratory, University of Oxford, and [2]Laboratoire de Cristallographie et de Cristallogenese des Proteines, Institut de Biologie Structurale,*

Hydrogenases offer important inspiration for future energy technologies where $H_2$ is set to play a major role. Reactions of the [FeFe]-hydrogenase from *Desulfovibrio desulfuricans* have been investigated by protein film voltammetry on a pyrolytic graphite ‘edge’ electrode. The studies reveal the conditions (potentials and kinetics) that control reversible inactivation under anaerobic oxidizing conditions and inhibition by CO and $O_2$. They also allow important comparisons with [NiFe]-hydrogenases.

It is thought that the anaerobically oxidised inactive state contains an extra $H_2O$ ligand, bound to the distal Fe in the active site[1]. The anaerobic reactivation process has been investigated over a range of pH values and electrode potentials using the electrochemical techniques of cyclic voltammetry and chronoamperometry. This approach has revealed that reactivation proceeds via a one electron transfer that is followed by a chemical reaction likely to be loss of the terminal water ligand.

Further studies have established that the reaction of O2 with the active site is highly potential dependent and not always irreversible. The higher the potential at which O2 is injected, the faster the rate of inactivation and reactivation, and the greater the percentage of recovered activity, suggesting that there are two possible reaction pathways, and that aerobic inactivation at high potential may generate the same species as anaerobic inactivation.

Exogenous CO binds strongly to active forms of the enzyme: however, CO binding is reversible, and the enzyme recovers as CO is lost from the solution. Activity is recovered most rapidly at low potential, suggesting that the most reduced form of the enzyme binds CO less strongly. The anaerobically oxidised inactive state is not inactivated by either CO or O2, rationalising observations of ‘protection’ of the hydrogenase that have appeared in early literature[2].

[1] Nicolet, Y. *et al. JACS* **2001**, *123*, 1596-1601

[2] C. van Dijk, A. van Berkel-Arts and C. Veeger, *FEBS.* **1983**, *156*, 340-344