Biological Hydrogen Activation is Cooperative: one H_2 activates the dinuclear center and a second H_2 is reduced

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The question "where does the substrate bind?" is a recurring theme in the biological activation of diatomics such as H_2, CO, N_2, whose simple structures sharply contrast with that of their biocatalysts. Long term attempts to determine the actual binding mode are typically based on data from crystallography, pre-steady-state kinetics, quantum-chemical calculations, and multiple spectroscopies. A consensus view for the binding of molecular hydrogen to its enzyme, hydrogenase, is yet to emerge.

There are two distinct classes of hydrogenases: NiFe- and FeFe-hydrogenases, each characterized by a distinctive functional core which is conserved within each class. The FeFe-hydrogenase of the strict anaerobic, mesophilic, sulphate reducing bacterium *Desulfovibrio vulgaris*, substrain Hildenborough, exhibits the highest specific H_2-production activity of all known hydrogenases, it is the only Fe-hydrogenase that can be purified aerobically in an O_2-insensitive, 'resting', form; remarkably, the kinetics of the hydrogen oxidation by *D. vulgaris* Fe-hydrogenase appears to have never been described in the literature.

Methyl viologen was used as a redox partner for steady-state kinetics on hydrogen oxidation and was found to obey classical one-substrate Michaelis-Menten kinetics. This simple one-substrate Michaelis-Menten kinetics was not found for hydrogen.

It is generally assumed that maximally only a single hydrogen/hydride interacts with the active site at any time. On the basis of results from the steady-state kinetics of hydrogen-oxidation we propose here a paradigm change: the FeFe-hydrogenase activity of the bacterium *D. vulgaris* requires the binding of two separate hydrogen molecules: a positive modulator and a substrate.