

## Exploring the reactivity of CO dehydrogenases

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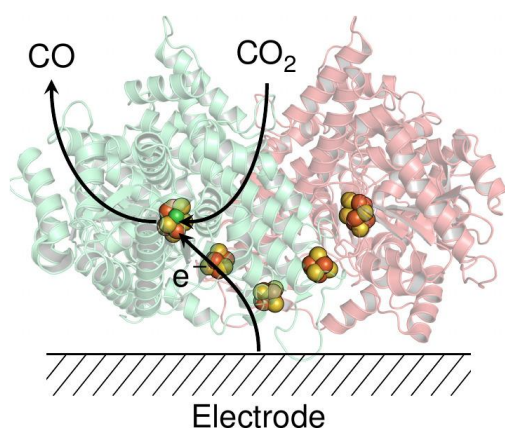
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NiFe CO dehydrogenases (CODHs) are metalloenzymes that catalyze the reversible reduction of CO<sub>2</sub> to CO at a NiFe<sub>4</sub>S<sub>4</sub> active site. They are very fast, with rates of CO oxidation counting in tens of thousands per second, and reversible, able to perform the conversion with very little driving force. Only a handful of CODHs, with high sequence similarity, have been studied in depth so far. However, in spite of this high similarity, their properties were found to be widely different, with K<sub>m</sub> values for CO oxidation ranging from 20 nM to 30 μM, and a large variability for K<sub>m</sub>s for CO<sub>2</sub>, bimolecular rates of reaction with O<sub>2</sub> spanning more than 2 orders of magnitude, and a large variation in their respective resistance to inactivation upon exposure to O<sub>2</sub>. We have investigated a number of different CODHs using a combination of Protein Film Electrochemistry<sup>1</sup>, biochemical techniques and crystallography. We have uncovered the exceptional resistance of the CODH from *D. vulgaris* to O<sub>2</sub><sup>2,3</sup>, linked to the formation of an alternative form of the active site<sup>4</sup>, demonstrated that CO oxidation from CODH IV from *C. hydrogenoformans* is diffusion-limited<sup>5</sup> and characterized the first CooS-type archeal CODHs<sup>6</sup>.



**Figure:** schematic representation of a CODH immobilized onto an electrode for studying using protein film electrochemistry

<sup>1</sup> del Barrio, M. *Acc. Chem. Res.*, **2018**, 51, 769-777

<sup>2</sup> M. Merrouch, et al, *Chem. Eur. J.*, **2015**, 21 (52), 18934–18938

<sup>3</sup> E. C. Wittenborn, et al, *ACS Catal.*, **2020**, 10, 7328-7335

<sup>4</sup> E. C. Wittenborn, et al, *eLife*, **2018**, 7

<sup>5</sup> L. Domnik et al, *Angew. Chem. Int. Ed. Engl.*, **2017**, 56 (48), 15466–15469

<sup>6</sup> M. Benvenuti et al, *Biochim Biophys Acta Bioenerg*, **2020**, 1861 (7), 148188