Structural insights on the mechanism of the electron-bifurcating [FeFe] hydrogenase from *Thermotoga maritima*

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Electron bifurcation is an important mechanism of energy conservation in nature but is still poorly understood. The electron bifurcating/confurcating [FeFe] hydrogenase from *Thermotoga maritima* (HydABC) simultaneously oxidises both NADH and ferredoxin and reduces protons generating hydrogen. How this enzyme operates is completely unknown, and the sites of ferredoxin binding and electron bifurcation/confurcation are under debate. Here, we present the 2.3 Å structure of HydABC solved by electron cryomicroscopy as an important step towards understanding its mechanism. The entire structure is a heterododecamer, $Hyd(ABC)_4$, composed of two independent $Hyd(ABC)_2$ 'halves' each made of two strongly interacting HydABC trimers electrically connected via a histidine-ligated [4Fe-4S] cluster in HydA (Fig. 1A). Using symmetry expansion we identified two conformations of the C-terminal domain (CTD) of HydB: a "closed bridge" conformation with the CTD of HydB bridging to an adjacent trimer, and an "open bridge" conformation with the CTD of HydB pointing away from the complex (Fig. 1B). This "bridge" structure is highly conserved in known bifurcating [FeFe] hydrogenases and is likely to be crucial for the mechanism. Furthermore, we identified a Zn^{2+} site connecting the CTD of HydB to the rest of HydB, which may act as a "hinge" regulating the flexibility in this region. These results point to a novel mechanism of electron-bifurcation and allow targeted studies of the mechanism of this fascinating enzyme.

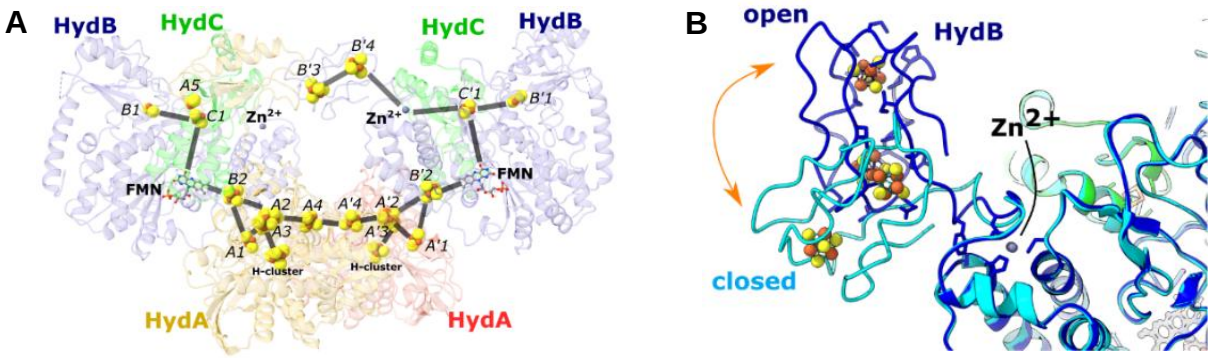

Figure 1. A) An atomic model of one $Hyd(ABC)_2$ hexamer with the HydB bridge domain in the closed position. B) Zn^{2+} hinge region, showing the two possible conformations of the HydB bridge domain, open (blue) and closed (light blue).