Molecular Dynamics Investigation of O_2 Diffusion Mechanisms in the Protective CbA5H Hydrogenase

Mattia P. Fossati[1], Jacopo Vertemara[1], Federica Arrigoni[1], Giuseppe Zampella[1], Luca Bertini[1], Luca De Gioia[1]

E-mail: m.fossati28@campus.unimib.it

[1] Department of Biotechnology and Biosciences, Univ. degli Studi Milano-Bicocca, Milano, IT

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Abstract: [FeFe]-hydrogenases are efficient biocatalysts for hydrogen conversion but are generally very sensitive in the presence of O_2; no [FeFe]-hydrogenase are known to function in the presence of O_2, some are reversibly inhibited (O_2-sensitive), while others are irreversibly destroyed (O_2-labile).

The *Clostridium beijerinckii* [FeFe]-hydrogenase (CbA5H) shows an O_2-protecting mechanism that is unique, forming an inactive but rapidly reversible state upon O_2 exposure. Experimental data suggests that the TSC-loop adopts a conformation that allows the cysteine residue (C367) to complete the electronic configuration of the distal iron atom in the H-cluster. However, the mechanism by which O_2 molecules reach the active site is less known. In this work we aim to investigate this process using advanced molecular dynamic simulations including unbiased molecular dynamic and random accelerated molecular dynamics (RAMD) to explore possible diffusion pathways within the protein. The RAMD technique will provide insights the timescales and diffusion rates associated to O_2 migration toward the active site (H-cluster) or [4Fe-4S]-clusters, contributing to a deeper understanding of the CbA5H's protective mechanism against O_2 damage.

The insights gained may support protein engineering effort for developing biocatalytic applications of O_2-tolerant [FeFe]-hydrogenases for sustainable H_2 production.