From the catalytic mechanism of the glycyl radical enzyme pyruvate formate-lyase to the dynamics of its activation

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Pyruvate formate-lyase (PFL) is a glycyl radical enzyme (GRE) that reversibly converts pyruvate and coenzyme A (CoA) to formate and acetyl-CoA in two half-reactions. PFL has a crucial role in the central glucose metabolism of *E. coli* and other microbes when switching to anaerobic conditions. [1] Due to their versatile functionalities, GREs have potential applications in biochemical and enzyme engineering. [2] Recently, it has been proposed that GREs also have a vital role in the numerous metabolic and biosynthetic pathways and environment due to their high abundance in the human gut microbiota.

The reactions catalyzed by GREs involve an extremely reactive and short-lived glycyl radical intermediate, which makes them very challenging for experimental studies. However, molecular modeling methods, based on classical and quantum chemical approach, provide valuable tools for the successful studies of these type of biomolecular systems.

Certain conformational changes of PFL and the entry of CoA in the active site are crucial for the second half-reaction to take place. Recently we proposed through extensive molecular dynamics (MD) simulations that the approach of CoA near the active site occurs through the newly appearing “open” state of the identified channel. [3] Further, by employing enhanced sampling methods we have shown that CoA is more likely to reside inside the active site after the first half-reaction whereby we characterized the stable and potentially reactive binding poses of CoA in the active site. [4]

Using full enzyme as a model for QM/MM calculation we explored the mechanism of both half-reactions. We confirm the progression of the first half-reaction with pyruvate in two steps without CoA occupying the active site. Further, we propose two-step mechanism for the second half-reaction with CoA. In addition, while a definite possibility of H-abstraction from CoA in the active site before the first half-reaction exists, we propose that this would cause a premature quenching of the radical and could ultimately lead to the inactivation of PFL.

In order to better understand the activation of GREs we explore the conformational dynamics of several representative GREs and the prototypical member of the radical SAM enzyme, namely pyruvate formate-lyase activating enzyme (PFL-AE). In this respect we performed preliminary multiple atomistic unrestrained MD simulations ranging to the timescales of several microseconds to uncover the connection between the fundamental conformational dynamics of GREs and the potential effects of their binding on the dynamics of the activating enzyme.

[1] L. R. F. Backman, M. A. Funk, C. D. Dawson, C. L. Drennan, *Crit. Rev. Biochem. Mol. Biol.*, **2017**, *52*, 674-695.
[2] C. M. Jäger, A. K. Croft, *Chem. Bio. Eng. Rev.*, **2018**, *5*, 143-162.
[3] M. Hanževački, K. Čondić-Jurkić, R. D. Banhatti, A-S. Smith, D. M. Smith, *Chem. Eur. J.*, **2019**, *25*, 8741-87535.
[4] M. Hanževački, R. D. Banhatti, K. Čondić-Jurkić, A-S. Smith, D. M. Smith, *J. Phys. Chem. A*, **2019**, *123*, 9345-9356.