Towards Engineering Radical Enzymes - Thermodynamic Reaction Profiling and Mechanistic Insights into QueE

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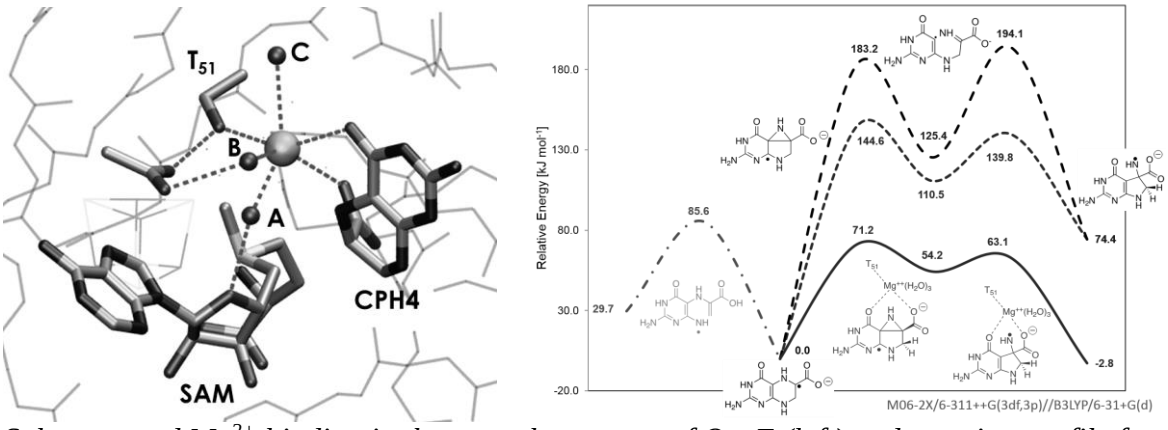
Radical S-adenosylmethionine (SAM) dependent enzymes[1] are a class of enzymes dealing with radical intermediates during catalysis. The enzymes harness these intermediates, which are hard control in classical synthesis, in a very controlled way for a wide range of challenging chemical processes leading to products of potential use in anti-viral, anti-cancer and antibiotic treatments. This makes them particularly attractive for enzyme engineering with its key goal to design enzymes for industrial biotechnological applications with improved or new properties which extend the chemistry of natural enzymes.

A thorough knowledge of the reaction mechanisms involved in the biocatalysis of these enzymes can lay the foundation for rational enzyme engineering. On the other hand this also shows one of the bottlenecks for a more rapid access to rational enzyme design. At best, all factors influencing the enzyme kinetics from substrate binding, the catalytic mechanism to effects by flexible protein dynamics are known in detail. Still, individual steps of the catalysis can be addressed by quicker methods, in order to get a first qualitative picture of how these steps can be influenced and manipulated which can feed into the enzyme design process.

Radical stabilization energies (RSEs)[2] for example, offer an attractive possibility to assess the overall thermodynamics of radical rearrangements as central steps in radical SAM enzyme catalysis. Through the example of the recently structurally resolved bacterial 7-carboxy-7-deazaguanine (CDG) synthase (QueE),[3] we will highlight key features and details of the biocatalytic reaction mechanism involved[4] and will investigate the potential of using radical stabilisation energies for of a rapid reaction profiling directly out of molecular-dynamics simulations of the enzyme substrate complex. Further, we will provide insights into other challenges of radical SAM enzymes addressed in the context of enzyme engineering.



Substrate and Mg^{2+} binding in the crystal structure of QueE (left) and reaction profile for radical rearrangement for model system (right).

[1] Broderick, J. B. et al., *Chem. Rev.*, **2014**, *114*, 4229-4317.
[2] Hioe, J. and Zipse, H.,.*Faraday Discussions*, **2010**. *145*: p. 301-313.
[3] Dowling, D. P. et al., *Nat. Chem. Biol.*, **2014**, *10*, 106-112.
[4] Jäger, C. M., Croft, A. K., *Chem. Eur. J.*, **2017**, *23*, 953-962.