Conformational heterogeneity in enzymatic catalysis and evolution

Gyula Hoffka[a], Viktor Ambrus[a], Letif Mones[b,c] and Monika Fuxreiter[a]

[a] *MTA-DE Laboratory of Protein Dynamics, University of Debrecen,* [b] *Mathematics Institute, University of Warwick, United Kingdom,* [c 3] *Engineering Laboratory, University of Cambridge, United Kingdom*
fmoni@med.unideb.hu

De novo enzyme design has been a long-term dream for industrial applications, while still presents a challenge for computational methods. It requires a fundamental understanding of enzymatic catalysis and those evolutionary forces, which optimize catalytic rates [1]. We explored the links between conformational plasticity and enzyme evolvability using multiscale and coarse-grained approaches [2]. We simulated the laboratory evolution of two Kemp eliminase designs (KE07 and HG3.17) and have demonstrated that optimization of reorganization energy is a major driving force of catalytic improvements [1-3]. At the same time, we identified multiple catalytically relevant configurations, and showed that conformational heterogeneity persists throughout the evolutionary trajectories. In particular, we demonstrated that simultaneous presence of alternative rotamers in HG3.17 are required to reproduce the experimentally observed barriers. We further showed that catalytic rates are robust to significant changes in the conformational ensemble; which promotes reducing promiscuous activities. We relate conformational diversity to co-evolving dynamical couplings [4], and propose that these two phenomena jointly shape the functional repertoire of enzymes in the cellular environment [5].

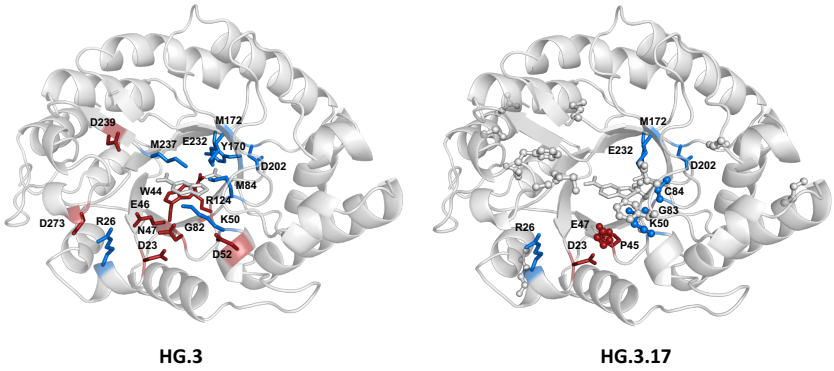

Figure 1: Residues with significant contributions to reorganization energy in the designed (HG.3) and evolved (HG.3.17) Kemp eliminase. Unfavorable contributions (λ ≥ 0.5 kcal/mol) are displayed in red, favorable contributions (λ < -0.5 kcal/mol) are in blue.

References

1. M. Fuxreiter and L. Mones, *Curr. Opin. Chem. Biol.* **21** (2014), 457.
2. M. Fuxreiter and L. Mones *The Empirical Valence Bond Approach as a Tool for Designing Artificial Catalysts* (2017).
3. A Labas, E Szabo, L. Mones and M. Fuxreiter *Biochim. Biophys. Acta* **1834** (2013) 908.
4. F. Zsolyomi, V. Ambrus and M Fuxreiter *Structure* (2019).
5. H. Wu and M. Fuxreiter, *Cell* **165** (2016), 1055.