Conferring natural enzymes new synthetically useful functionalities

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Enzymes are proteins that catalyze chemical reactions essential to the survival of living organisms. Nature has evolved these catalysts over millions of years to provide substantial reaction rate acceleration and remarkable specificity towards a single desired product. The ability to design novel enzymes that catalyze chemical reactions that do not exist in nature would have a profound impact on numerous fields such as biotechnology, biomedicine, and defense against chemical and biological warfare.

Many experimental Directed Evolution (DE) studies have been published where the catalytic activity of an enzyme has been changed, which range from single mutations to deletion and substitution of loops in addition to amino-acid substitutions. The main drawback of DE is that still tens of thousands of variants need to be tested experimentally. In addition to that, little information is available as to how these mutations lead to enhanced enzyme proficiency. The understanding of how DE operates in these cases is of extreme relevance for the improvement of the existing inside-out computational protocol for designing enzymes. Despite the initial successes of the protocol, our ability to improve enzyme activity towards a desired reaction in a rational manner has remained underdeveloped. The most active computationally designed enzymes still perform quite poorly in comparison with the natural and DE-engineered enzymes.

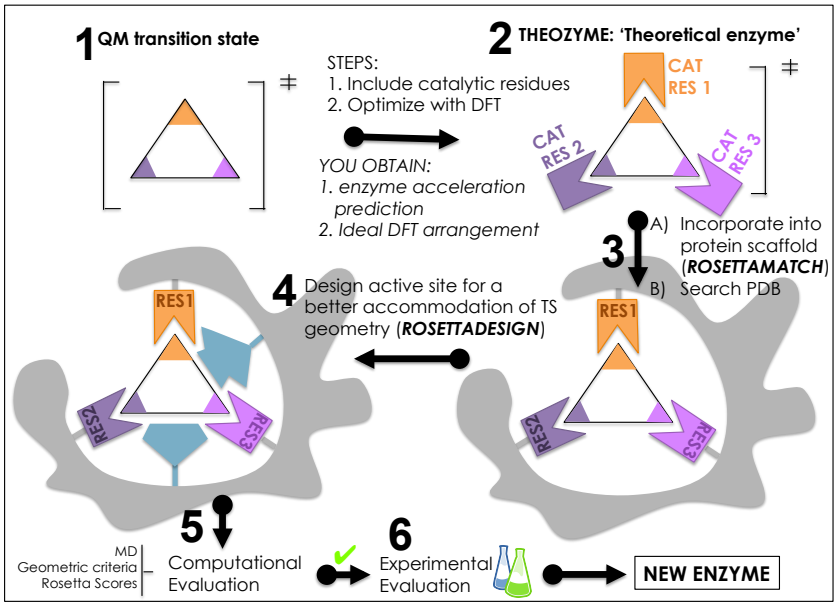

Figure 1. Representation of the inside-out protocol

Jiménez-Osés, G.; Osuna, S.; Gao, X.; Sawaya, M.R.; Gilson, L.; Collier, S. J.; Huisman, G.H.; Yeates, T.O.; Tang, Y.; Houk, K.N., Nature Chem. Biol. **2014**, *10*, 431-436.

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