Engineering protein dynamics for the understanding of divergent evolution of the Renilla luciferase

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Current protein engineering methods typically use amino acid substitutions to improve protein activity, stability or solubility. However, to alter the protein function profoundly or even develop a novel function, substitutions might not be sufficient. In the present study, we employed the methodology of insertion and deletion (InDel) mutagenesis (Previously published in: Emond S et al. (2019) BioRxiv) which allows more significant changes in a protein backbone conformation and dynamics, while it might also lead to the collapse of the structure. To compensate for the disruptive effect of the methodology, we applied the InDel mutagenesis on a recently reconstructed hyperstable ancestor of two structurally similar but functionally different enzymes, haloalkane dehalogenases and coelenterazine-dependent *Renilla*-type luciferases (Previously published in: Chaloupkova R et al. (2019) ACS Catalysis 9, 4810–4823). Screening of the generated libraries led to the identification of three distinct protein regions, whose alteration resulted in a markedly improved luciferase activity. Crystallographic analysis of the best InDel variant identified open and closed conformations, introduced by the mutagenesis. Transient kinetics revealed a two-step binding of the bulky substrate and enhanced substrate affinity, both linked to the altered dynamics. The hydrogen-deuterium exchange experiments and molecular dynamics simulations confirmed increased flexibility of the identified regions. Our multidisciplinary approach underlines the significance of dynamics for the evolution of the luciferase activity and proposes the putative evolutionary divergence pathway from hydrolytic dehalogenation to oxidative bioluminescence. The results also demonstrated the potential of InDel mutagenesis for a modification of protein dynamics and the development of novel catalytic activities.

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