ENZYME EVOLUTION AND ENGINEERING USING INSERTIONS AND DELETIONS

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In Nature, proteins evolve and acquire new functions by accumulating mutations. Substitutions and InDels (Insertions and Deletions), as well as circular permutations and rearrangement of protein domains, account for the majority of evolutionary changes. While the effects of substitutions have been extensively studied and documented, understanding the structural and functional effects of InDels still remains a challenge. InDels are assumed to be highly deleterious mutations because they are more likely to disrupt the structural integrity of proteins than are substitutions. On the other hand, they may induce significant structural changes that substitutions alone cannot cause and thus are believed to be key players in many natural evolutionary processes, such as the modification of active site loops to generate new enzyme functions[1] or the emergence of new protein structures[2].

We aimed at performing directed evolution by randomly incorporating InDels to investigate how they would be tolerated and whether they could be selected for functional improvements. Starting from a previously reported methodology[3], we developed a library construction approach to randomly incorporate InDels within a DNA sequence of interest and applied it to generate InDel variant libraries of a promiscuous enzyme (phosphotriesterase[4]). We screened the resulting libraries (i) to compare the impact of InDels to that of substitutions on the enzyme, (ii) to identify adaptive InDels improving a new (or promiscuous) activity and (iii) to investigate the interaction between InDels and substitutions in an adaptive process. Our results show that, while being generally more deleterious than substitutions, InDels can also lead to functional improvements and may allow access to alternative evolutionary trajectories.

References
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