530g Laboratory Evolution of a Fluorinated Protein

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Directed evolution was utilized to recover the activity of chloramphenicol acetyltransferase (CAT), after fluorination of the enzyme by incorporation of 5,5,5-trifluoroisoleucine (5TFI). When 5TFI was incorporated into wild-type CAT, replacing all nine isoleucine residues, k_{cat}/K_m was reduced from 4.08 ± 0.4 to 2.03 ± 0.2 x 10^4 μM^{-1} min^{-1}. Through the use of error-prone PCR and a selection scheme that relies on the expression of functional CAT mutants containing 5TFI, we found a fluorinated mutant with activity similar to that of the conventional wild-type enzyme (k_{cat}/K_m= 3.65 ± 0.4 x 10^4 μM^{-1} min^{-1}). This mutant contains a single mutation at position 61, H61Y, which is positioned between two 5TFI residues at positions 60 and 62. In all cases, the incorporation level of 5TFI was confirmed to be over 83% based on MALDI-TOF mass spectrometry and amino acid analysis. This result indicates that directed evolution can complement the incorporation of non-canonical amino acids as a tool in the engineering of novel proteins.