

DIRECTED EVOLUTION OF A FLUORINASE FOR IMPROVED FLUORINATION EFFICIENCY ON A NON-NATIVE SUBSTRATE

Huihua Sun, Metabolic Engineering Research Laboratory (MERL), Agency for Science, Technology, and Research (A*STAR)
sunhh@merl.a-star.edu.sg

Fluorinases offer an environmentally friendly alternative for selective fluorination under mild conditions. However, their diversity is limited in nature and they have yet to be engineered *via* directed evolution. Herein, we report the directed evolution of the fluorinase FIA1 for improved conversion of a non-native substrate, 5'-chloro-5'-deoxyadenosine (5'-CIDA), to 5'-fluoro-5'-deoxyadenosine (5'-FDA). The evolved variants, fah2081 (A279Y) and fah2114 (F213Y, A279L), were successfully applied in the radiosynthesis of 5'-[¹⁸F]FDA, with overall radiochemical conversion (RCC) >3-fold higher than FIA1. Kinetic studies of the 2-step reaction revealed that the variants significantly improved k_{cat} in the conversion of 5'-CIDA to S-adenosyl-L-methionine (SAM) but decreased k_{cat} in the conversion of SAM to 5'-FDA.