Engineering intracellular enzyme localization and pathway biocatalysis

Ian Wheeldon
Chemical and Environmental Engineering
University of California, Riverside

Many essential and industrially useful metabolic pathways are assembled on intracellular membranes or are sequestered in organelles and protein microcompartments. Connections and organization between enzymes in these structures produce optimized kinetics through controlled local concentrations of enzymes and pathway intermediates. When engineering new pathways such organizations and intracellular localizations are often lost, thus eliminating any kinetic advantages that may have resulted. We have discovered this to be true with ester biosynthesis pathways engineered to produce fragrance and flavor compounds, solvents, and biofuels. The key reaction step, acetyl transfer from acetyl-CoA to an alcohol, localizes to the endoplasmic reticulum and lipid droplets in yeast and disruption of this localization significantly limits enzymatic activity. By first understanding how alcohol-O-acetyltransferases (AATases) from yeast and fruit species express and localize, we have developed engineering solutions that overcome the losses in activity due to disrupted intracellular localization. When highly active AATases are heterologously expressed in *E. coli* activity is decreased by nearly three orders of magnitude from levels observed in native hosts. Controlling expression level and preventing aggregate formation partially recovers the lost activity. An alternative solution is to introduce new multienzyme structures that create spatially organized metabolic pathways around the native intracellular localization of highly active AATases. Co-localization of upstream enzymes to lipid droplets locally synthesizes pathway intermediates, producing kinetic enhancements in the AATase pathway and increased fermentation titers. The solutions developed for ester biosynthesis were designed to be broadly applicable and promise to help solve challenges in the disruption of native enzyme localization and pathway structure that limit the engineering of other potentially useful pathways.

Bio: Dr. Wheeldon is an Assistant Professor of Chemical and Environmental Engineering at the University of California, Riverside. He received his PhD in Chemical Engineering from Columbia University in 2009, and completed two years of postdoctoral training at Harvard Medical School and the Wyss Institute for Biologically Inspired Engineering at Harvard University. Dr. Wheeldon received a Master's of Applied Science from the Royal Military College of Canada (2003), and a Bachelor's of Applied Science (1999) from Queen's University, Canada. His research is focused on protein and biomolecular engineering for biocatalysis and metabolic engineering.