

Modeling Biocatalyst Development - Bommarius

Improving directed evolution through pooling

Pooling in directed evolution experiments will greatly increase the throughput of screening systems, but important parameters such as the number of good mutants created and the activity level increase of the good mutants will depend highly on the protein being engineered. We developed and validated a Monte-Carlo simulation model of pooling that allows the testing of various scenarios in silico before starting experimentation. Using our test enzymes, -galactosidase (supermutant, or greatly improved enzyme) and -glucuronidase (dud, or enzyme with ancestral level of activity), the model accurately predicted the number of supermutants detected in experiments within a factor of 2. Pooling is most suited to cases such as the directed evolution of new function in a protein, where a large increase in activity over the background level will occur. Pooling can be used when new mutants have a lower level of activity increase, if a very sensitive assay is employed. Using our model will increase the throughput of screening procedures for directed evolution experiments and thus, lead to speedier engineering of proteins.

