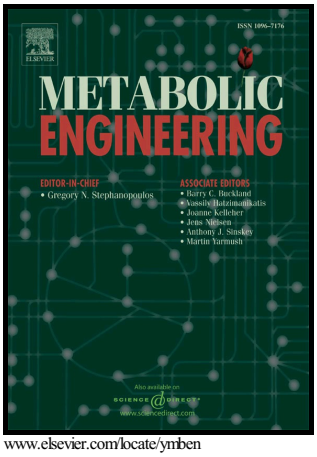


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# A cell-free framework for rapid biosynthetic pathway prototyping and enzyme discovery

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## Abstract

Speeding up design-build-test (DBT) cycles is a fundamental challenge facing biochemical engineering. To address this challenge, we report a new cell-free protein synthesis driven metabolic engineering (CFPS-ME) framework for rapid biosynthetic pathway prototyping. In our framework, cell-free cocktails for synthesizing target small molecules are assembled in a mix-and-match fashion from crude cell lysates either containing selectively enriched pathway enzymes from heterologous overexpression or directly producing pathway enzymes in lysates by CFPS. As a model, we apply our approach to *n*-butanol biosynthesis showing that *Escherichia coli* lysates support a