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Commentary

Protein Engineering Impending Implications in Synthetic Biology

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DESCRIPTION

Over decades, protein engineering has been a strong tool in biotechnology for producing a large variety of useful enzymes for industrial uses. Today, protein engineering plays a critical role in developing the emerging science of synthetic biology, because metabolic engineering efforts alone are insufficient to realize synthetic biology's full potential. The discussion in this article is on improvements in protein engineering approaches for increasing biocatalytic capabilities to optimize designed pathways in host systems, which are important for achieving high titer production of target molecules. The precise ways in which protein engineering has boosted metabolic engineering efforts, as well as our judgment of its potential to further advance biological engineering as a whole synthetic biology is a vast field of study that blends biology and engineering to create new products. Synthetic biology is a large field of study that combines biology and engineering to design and build biological systems with novel functions not present in nature. Although the term "synthetic biology" was coined in 1980, the study field's potential is only now being recognized.

The development of new metabolic pathways to produce a nonnative target product frequently encounters the obstacle of low product yield due to low enzyme activity in the synthetic route, which may be related to non-ideal circumstances in heterologous systems. Rather than merely increasing the protein expression level to compensate for the low activity, it would be more useful for energy and resource conservation to tailor the enzymes for better *in vivo* activity while keeping a modest expression level. Increase particular enzymatic activities in the production host under the needed production conditions are one technique to improve *in vivo* activities of enzymes for synthetic biology. For instance, the hyperthermophilic Geranylgeranyl Diphosphate (GGPP) Synthase (GPS) from *Archaeoglobus fulgidus* has been optimised by directed evolution to function at ambient temperature for increased production of GGPP required for the biosynthesis of the antioxidant astaxanthin in *E. coli via* the lycopene biosynthetic pathway.

Selected mutations were observed to improve astaxanthin precursor lycopene synthesis by 60-100%. Furthermore, ginkgolides, the active chemicals in herbal medicines isolated from *Gingko biloba* were shown to have potential uses in migraine treatment and delaying the progression of Alzheimer's disease.

This sparked interest in improving the production of the ginkgolides precursor, levopimaradiene, in *E. coli* . LPS from *Gingko biloba* was produced *via* site-saturation mutagenesis based on a structural model of the enzyme's second binding site, while GPS from *Taxus canadensis* was improved using directed evolution to alleviate bottlenecks in the metabolic process. Utilizing engineered LPS and GPS in a piece of technology, researchers were able to obtain a 2600-fold increase in levopimaradiene yield. Protein engineering has also been used to increase the bioproduction of Polyhydroxyalkanoate (PHA), a biopolymer found naturally in some bacteria strains. Directed evolution was employed to improve the specific activity of *Aeromonas punctata* PHA synthase in recombinant *E.coli* that synthesises the substrate (R)-3-hydroxybutyryl-CoA. The isolated PHA synthase variations accumulated up to 126 percent more PHA than the wild-type. This could have an industrial application in the production of biodegradable polymers. Increased functional expression of enzymes can also increase *in vivo* activity since inactive insoluble heterologous protein aggregates can form when their genes are over-expressed in many host systems. Humulene synthase (HUM) from *Abies grandis* is a highly flexible enzyme with potential applications in terpenoid bioproduction, but it misfolds and clumps in *E. coli*. The multiple sequence alignment of HUM with homologous enzymes was analyzed using a mathematical model based on an adaptive evolution mechanism. Glycine and proline residues were redistributed in the enzymes based on conservation probability of the two amino acids at each residue, and more than 80% of the predictions were correct.

Recombining the favorable mutations produced HUM versions that were more soluble and boosted sesquiterpene synthesis by an order of magnitude. Enzymatic activity enhancement is most likely the most prevalent goal of protein engineering efforts. Productivity can be increased in synthetic biology applications by enhancing specific activity to relieve bottlenecks in metabolic flow and enhance cell development by preventing the accumulation of harmful intermediates. Improving functional production of essential proteins that are insoluble in heterologous hosts, such as plant cytochromes P450, will speedup the development of designed metabolic circuits that rely on these enzymes. As a result, increasing enzyme activity in vivo is a critical tool for synthetic biology that should not be disregarded.

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