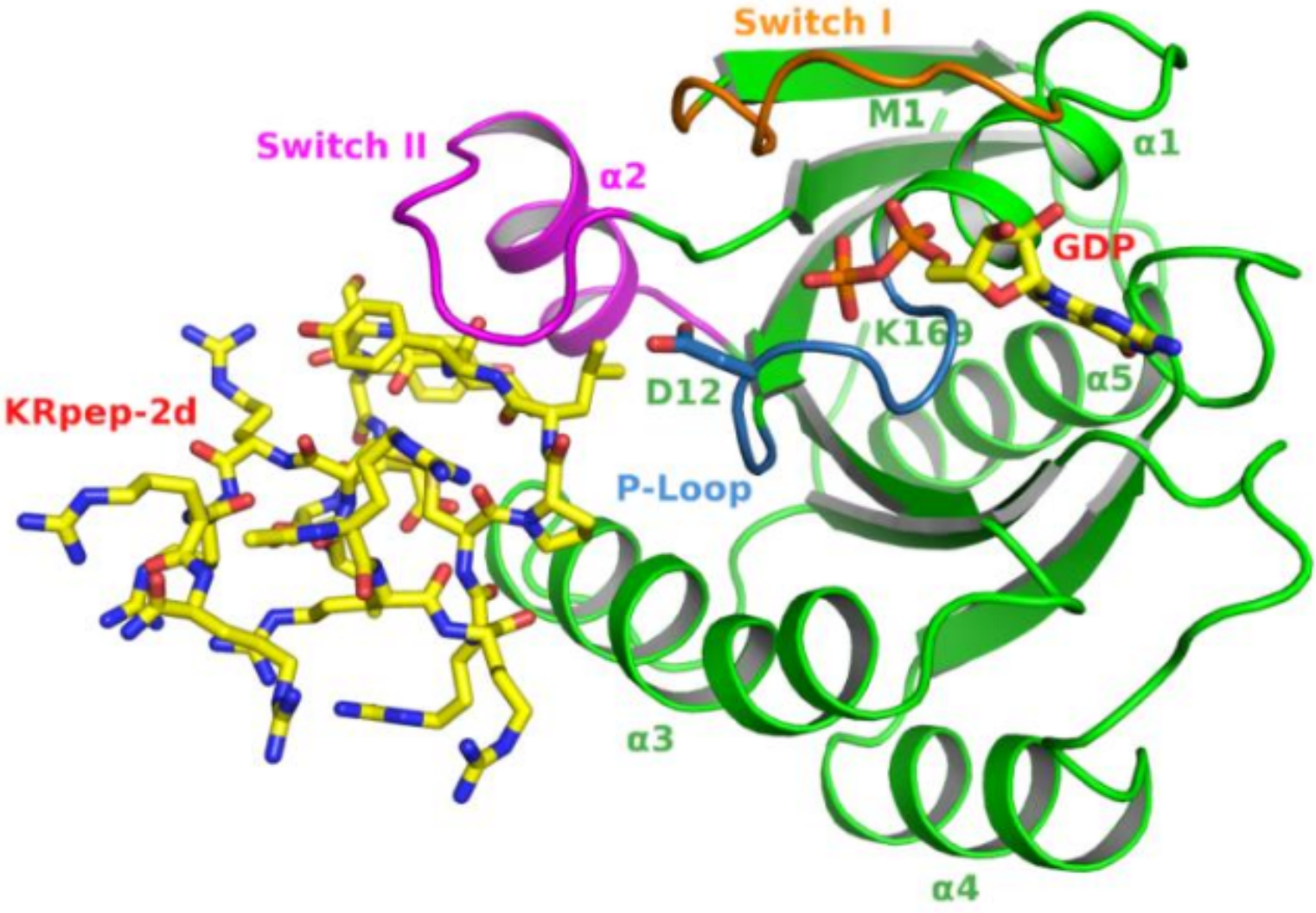


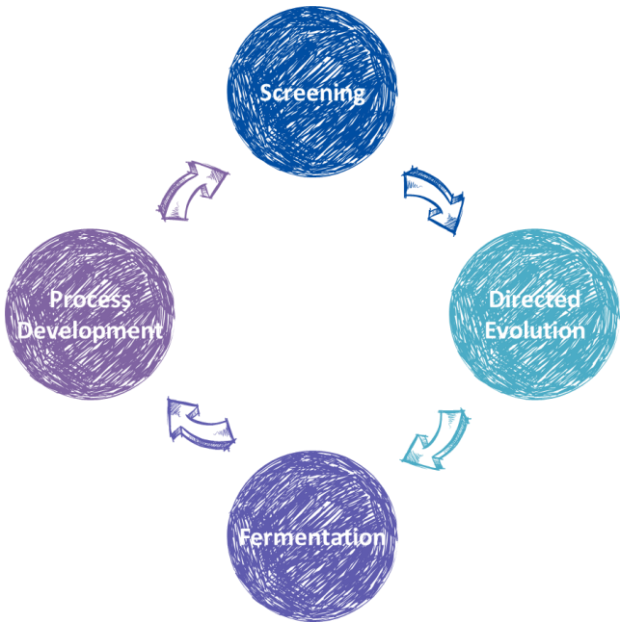
Biocatalysis Technology

Screening, Evolution, Fermentation & Process Development

November, 2021

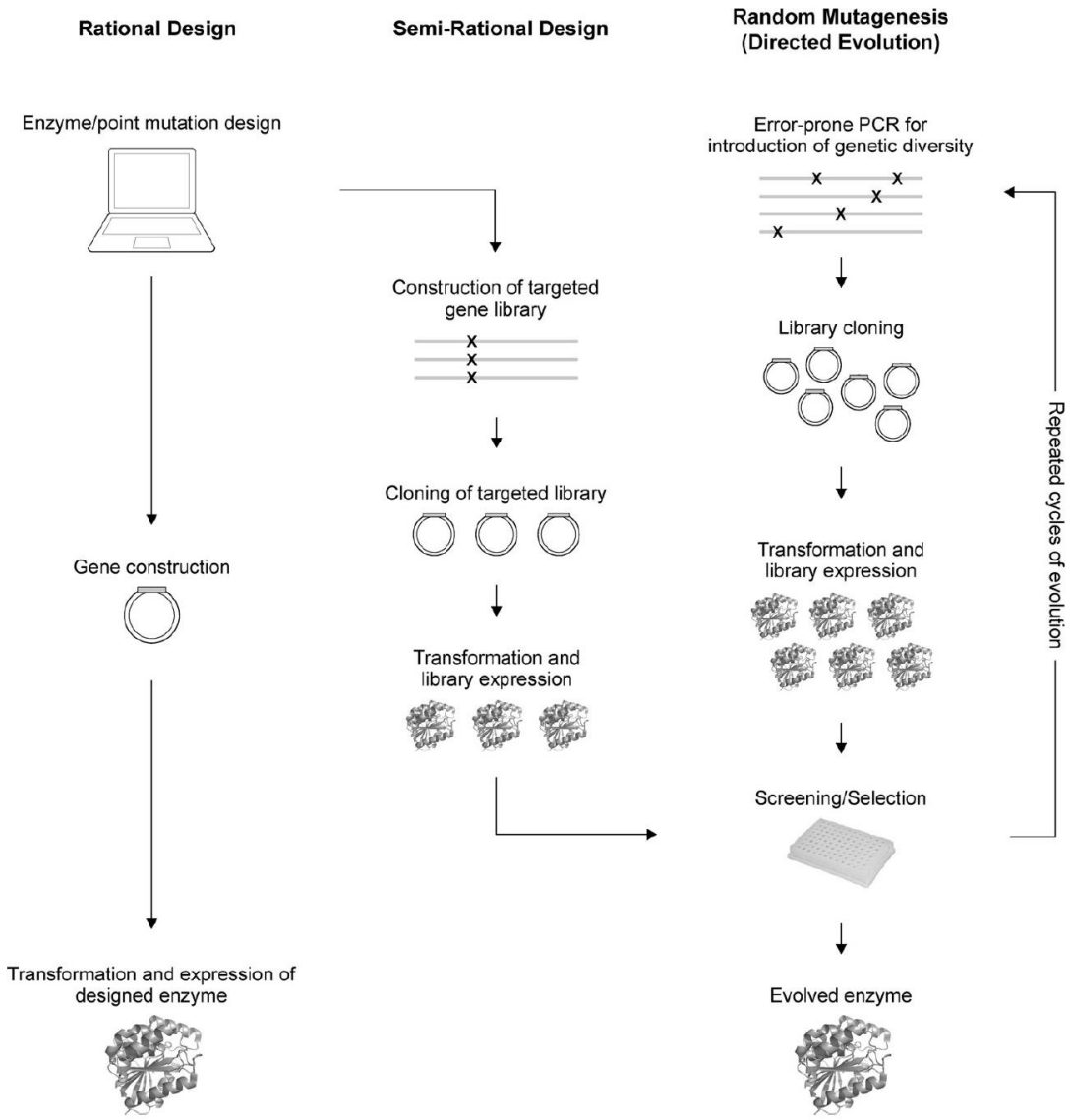
PharmaBlock

Pharmaceutical industry continuously looks for efficient, economic, and environmental-friendly technologies to advance pharmaceutical research and production. Among them, biocatalysis stands out due to its high selectivity (chemo-, regio-, and stereo-), mild reaction conditions and tunable catalysis properties. The streamline of enzyme screening, directed evolution, fermentation production of enzymes, and chemoenzymatic process development can facilitate the customized process and production of APIs and intermediates.



Currently the most widely used enzymes in small molecule synthesis include ketoreductases (KRED), tranaminases (ATA), lipase, nitrilases, and oxidases. Some other emerging enzymes are having increasingly more uses in industry, such as enereductases, iminereductases, and aminodehydrogenases. The arsenal of enzymes for small molecule synthesis are constantly expanding, and more types of chemical transformations are seeing their biocatalysis counterparts.

Besides the widening of enzyme species, enzyme engineering, specifically enzyme directed evolution offers an explicitly precise way to modify and improve the enzyme's performance in terms of reactivity, selectivity and pH or temperature compatibility etc. By introducing amino acid change, the evoluted enzymes can show modified properties. And by picking the improved ones for further rounds of evolution, scientists can direct the evolution to deliver better-performing enzymes. The design of the changed amino acids can root in random mutagenesis, semi-rational design and rational design (in the scheme below) with the first two being of practical value to the industry.



Referenced from Porter, J. L.; Rusli, R. A.; Ollis, D. L. Directed Evolution of Enzymes for Industrial Biocatalysis. *ChemBioChem* **2016**, *17*, 197−203.

Challenges

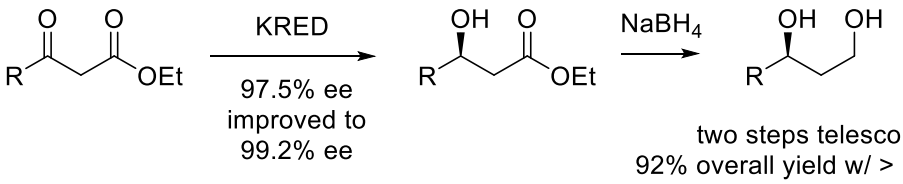
Although the roadmap is quite clear, there are several practical challenges in steaming up the streamline, specifically in CDMOs.

First, the satisfied enzyme are not trivial to obtain most times. Directed evolution process can take rounds after rounds and consumes tremendous time and resources, especially for random mutagenesis which can easily induce millions of mutants for screening. Time and resources are the top concerns in drug substance development. One way to relief is a smart enzyme stock which can either directly deliver a useful

enzyme, or give a close-to-success hit for further evolution. In this way, the evolution can usually rely on semi-rational design, which greatly saves time and resources.

Secondly, enzyme engineering service and CDMO service used to be separate businesses, and needed to be aligned case by case for customers' projects. However, barriers between the two sectors always exist, as the enzyme performance is largely influenced by substrate structures, reaction conditions, and quality control of the substrates. In addition, the pharmaceutical industry has its unique IP rules and CMC regulations from the traditional consumers of enzyme engineering services, eg. food and beverage companies, which frequently makes the alignment even harder. While this traditional mode can be tedious and inefficient, integration of enzyme engineering into CDMOs can lower the cost and shorten the time, and offer better one-stop shopping experience.

Case study: semi-rational evolution simultaneously improves the enzyme's activity and stereoselectivity



In this reaction, the step1 is a KRED catalyzed enantio-selective reduction of ketone, and the step2 uses $NaBH_4$ to furnish a second hydroxyl group. The initial hit in PharmaBlock's in-house enzyme stock gave 97.5% ee, with roughly 1% ketone remaining after 5 hours reaction. This screening outcome is very promising though the

needed improvements were instantaneously identified by the team. One is that the ee selectivity needs further improvement to at least 99.0%. The other is that the enzyme activity shall be higher as any remaining ketone will be reduced to alcohol with no selectivity in the step2 and deteriorate the ee. The enzyme's ee selectivity and activity must be sufficiently high at the same time.

Based on our previous knowledge and experience, we applied semi-rational evolution strategy on the initial hit. We designed and tested 20 evoluted enzymes, and fortunately identified one that simultaneously satisfy the above two improvement requirements. This dual task was completed efficiently in a single week, and subsequent enzyme fermentation and kilo-scale reaction were completed in another week. This case illustrates the power of PharmaBlock's smart enzyme stock and evolution strategy, and integrated expertise of biocatalysis and chemoenzymatic process development.

What makes PharmaBlock unique?

PharmaBlock holds an in-house enzyme stock, complemented with many well-testified commercial enzymes. The in-house enzyme stock keeps expanding from evolution of existing members and addition of novel species. What's more, the building block projects greatly help our biocatalysis capability development. During continuously optimizing PharmaBlock's enzyme-routed building blocks, the biocatalysis team and the enzyme stocks are being evoluted rapidly. We have showcased our well-practiced skills in many of our CDMO projects as well.

A glimpse into the future

The biocatalysis team has built a brand new fermentation kilo lab in the ready-to-operate Nanjing site. Manufacture-sized fermentation plant is also in the blueprint. By collaborating with our engineering and equipment team, we will apply fixed enzyme and innovated biocatalysis reactor shortly. Providing better, faster and cheaper biocatalysis solution to our customers is the goal of the team.

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