Editorial

The central aim of protein engineering is the efficient creation of novel and practical biocatalysts and to understand structure/function relationships. The ultimate goal would be to create proteins designed to order on the lab bench. An efficient protein engineering strategy is necessary to design enzymes with improved properties. The basic strategies for protein engineering include rational design and directed evolution approaches. Rational design methods, such as site-directed mutagenesis, has been met with limited success due to our incomplete knowledge of structure/function relationships. On the other end of the spectrum, directed evolution approaches rely on iterative cycles of random mutagenesis and/or recombination to create large libraries of variants that are coupled to an efficient selection or screening strategy for identifying mutants with improved performance. In this issue of *Combinatorial Chemistry & High Throughput Screening*, we have assembled a collection of review and research articles using directed evolution approaches for protein engineering.

The review by Rubin-Pitel and Zhao summarizes the recent achievements in biocatalyst engineering by directed evolution. The manuscript focuses on altering activity, selectivity, substrate specificity, stability, and solubility. The creation of novel enzyme activity and products are highlighted.

Library creation is an important aspect of directed evolution. The review by Wong *et al*. addresses the diversity challenge of how to generate unbiased gene libraries by random mutagenesis. The manuscript is a comprehensive survey that summarizes, categorizes, and compares the methods for creating genetic diversity. The review is particularly useful for research labs that do not have experience with directed evolution methods.

Gilbert proposes the exon theory of genes that suggests the first genes were composed of a combination of small polypeptide chains or blocks. The process of creating new genes and protein evolution is a fundamental question that may never be answered. The review by Tsuji *et al.* outlines their approach to create novel proteins by block shuffling. The authors explores the foldability and enzyme activity of mutants created by permutations of modules or secondary structural units. In order to create their libraries, a new DNA recombination approach was developed to access sequence space that is not accessible through conventional methods such as DNA shuffling or family shuffling. This contribution summarizes the strategy to create proteins by block shuffling and the possible applications.

A key for a successful directed evolution experiment is often the screening assay. Fluorescence activated cell sorting (FACS) is powerful high-throughput screening approach to isolate and identify mutants from large protein libraries. FACS has been applied successfully in isolating proteins with improved or altered binding affinity. However, FACS screening for mutants with enhanced catalytic active has been met with limited success. The review by Farinas focuses on the FACS screening of protein libraries for enzymatic activity.

Creating proteins that can specifically recognize a designed DNA sequence continues to be challenge. Duria *et al.* have developed two bacterial one-hybrid systems to examine and select for zinc-finger/DNA interactions *in vivo*. The one-hybrid system is composed of a plasmid containing the gene for the zinc-finger fused to a fragment of RNA polymerase, and the reporter plasmid has the punitive zinc-finger binding site upstream the reporter. The advantages and the appropriate applications for this system are discussed.

The cofactor requirement might limit the industrial applications for NAD(P)H-dependent oxidoreductases since the pyridine cofactors are very expensive. Hence, cofactor regeneration systems are viable solutions to this problem. Woodyer *et al.* used a combination of directed evolution approaches and rational design methods to optimize phosphite dehydrogenase for NAD(P)H regeneration.

High-throughput screens for enantioselective enzymes are oftentimes time-consuming, and eliminating inactive mutants with a pre-screen/selection may provide a more streamlined process. Reetz and Wang have developed a pre-selection to eliminate inactive epoxide hydroylase mutants. The selection is based on the ability of an active epoxide hydrolase to catalyze the hydrolysis of the toxic epoxide substrate.

Wong *et al.* have developed a simple and economical high-throughput pre-screen for hydrolase and dehydrogenase activity that is based on the detection of aldehydes. Hydrolases and dehydrogenases have industrial applications for the synthesis of optically active amines and alcohols. Furthermore, the reverse reaction also can be useful to generate amides or esters *via* transfer of acyl moiety from an acyl donor compound to an acceptor.

O'Loughlin and Matsumura have created a novel protease-activated reporter enzyme to screen for protease activity, and β-glucuronidase and alkaline phosphate were used as model systems. These enzymes were engineered to contain three peptides attached to the C-terminus of the proteins. The first contains a protease cleavage site which activates the enzyme. The next peptide contains an epitope to monitor expression, and the last peptide contains a random sequence of twelve amino acids. Screening mutant libraries of β-glucuronidase and alkaline phosphate identified variants that are activated upon peptide cleavage.

In conclusion, directed evolution is a reliable tool to improve properties of proteins which can be used for industrial, pharmaceutical, and biotechnological applications. Laboratory evolution is also being used to elucidate complicated structure/function relationships which will help build the "rules" or principles for protein design. Furthermore, it is becoming a general method to manipulate metabolic pathways, create new screening systems, and understand the natural process of evolution. In years to come, laboratory evolution approaches will be used routinely in research labs, and it might eventually be found as common experiments in undergraduate biochemistry laboratory courses.

Edgardo T. Farinas
Department of Chemistry and Environmental Science
New Jersey Institute of Technology
University Heights
Newark, NJ 07102
USA
E-mail: Farinas@adm.njit.edu