Fluorescence Activated Cell Sorting for Enzymatic Activity

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Abstract: Directed evolution is a reliable method for protein engineering and as a tool for investigating structure/function relationships. A key for a successful directed evolution experiment is oftentimes the screen. Fluorescence activated cell sorting (FACS) is powerful high-throughput screening approach to isolate and identify mutants from large protein libraries. FACS has been successful in isolating proteins with improved or altered binding affinity. However, FACS screening for mutants with enhanced catalytic activity has been met with limited success. This review focuses on the FACS screening of protein libraries for enzymatic activity.

Keywords: Directed evolution, FACS, high-throughput screening, enzyme engineering.

INTRODUCTION

The goal of engineering biomolecules is to design proteins with improved properties or novel activities. Directed evolution has emerged as the method of choice for protein engineering and as a tool for investigating structure/function relationships [1]. Laboratory evolution is an iterative process of creating gene libraries by random mutagenesis or recombination followed by a screen or selection to identify mutants with the desired property. One of most challenging aspects of a successful directed evolution experiment is developing a reliable high-throughput screen. Traditional methods for screening proteins are based on microtiter or agar plate assays, and these formats have a low- to medium-throughput capacity. Although, these systems can be automated only 10^3 – 10^5 mutants can be typically screened. Therefore, new high-throughput screening methods are becoming more important. Fluorescence activated cell sorting (FACS) is becoming a powerful method for selecting mutants from large libraries. This review will focus mainly on FACS screening of enzymes.

FACS BASED SCREENING

FACS has been used in the analysis of enzyme activity and sorting complex cell populations [2, 3], and it is quickly becoming an important protein engineering tool [4-6]. In short, a flow cytometer is an instrument that examines single cells or particles which have been lined up in a single file from a sample. The cells are then analyzed by a focused laser beam [7-9], and the amount of light shielded by cell opposite the laser beam and the amount of light reflected at a right angle to the incident beam are recorded. These measurements can detect changes in wavelength due to a fluorescent probe as well as the cell granularity and morphology. Flow cytometers have the ability to identify and isolate fluorescent cells at a rate of approximately 1 – 10 x 10^7 cells per hour. In order to isolate active enzymes using FACS, the cells expressing the mutants must retain a fluorescent product and inactive variants should have a low fluorescent background. The fluorescent product must be associated with the cell to establish a genotype/phenotype connection. In other words, a physical link must be established for activity and the gene coding for the protein. This method has been widely utilized for isolating proteins with altered or increased binding affinity. Isolating proteins with improved binding affinity can be achieved by tagging the substrate with a fluorophore, and the protein-substrate complex remains intact. For example, FACS screening of displayed antibodies has been successful in identifying variants with improved or altered binding affinities [6,10]. On the other hand, FACS screening for enzyme activity is technically more challenging since the product fluorophore must remain associated with the cell and not diffuse away. FACS screening of enzymes can be divided into three categories based on the location of the enzyme: 1) displayed on a membrane of a microbe, 2) retention inside the cell, and 3) compartmentalization in an emulsion.

FACS SCREENING OF DISPLAYED ENZYMES

An advantage of displayed enzymes is that they have direct access to substrates which no longer have to pass through cell membranes to interact with the protein. Furthermore, there is greater control of the reaction environment and conditions compared to the complex interior of a microbial cell. However, linking the activity with the code of the gene is one of the main hurdles. On the other hand, one disadvantage of displaying a protein on the cell surface is that viability may be compromised. As a result, cells containing the improved protein can be sorted, but they cannot be cultivated and isolated. This situation can occur if the cell lyses during the screening procedure or the substrate is toxic to the cells. Hence, the DNA sequence cannot be identified for the improved protein resulting in the possibility of missing the mutant.

The first report of using FACS for enzyme screening was to develop a quantitative method to identify enzymes with novel substrate specificity [11]. The serine protease OmpT from *Escherichia coli* was chosen and it resides on the outer membrane of the cell. The enzyme has a preference to

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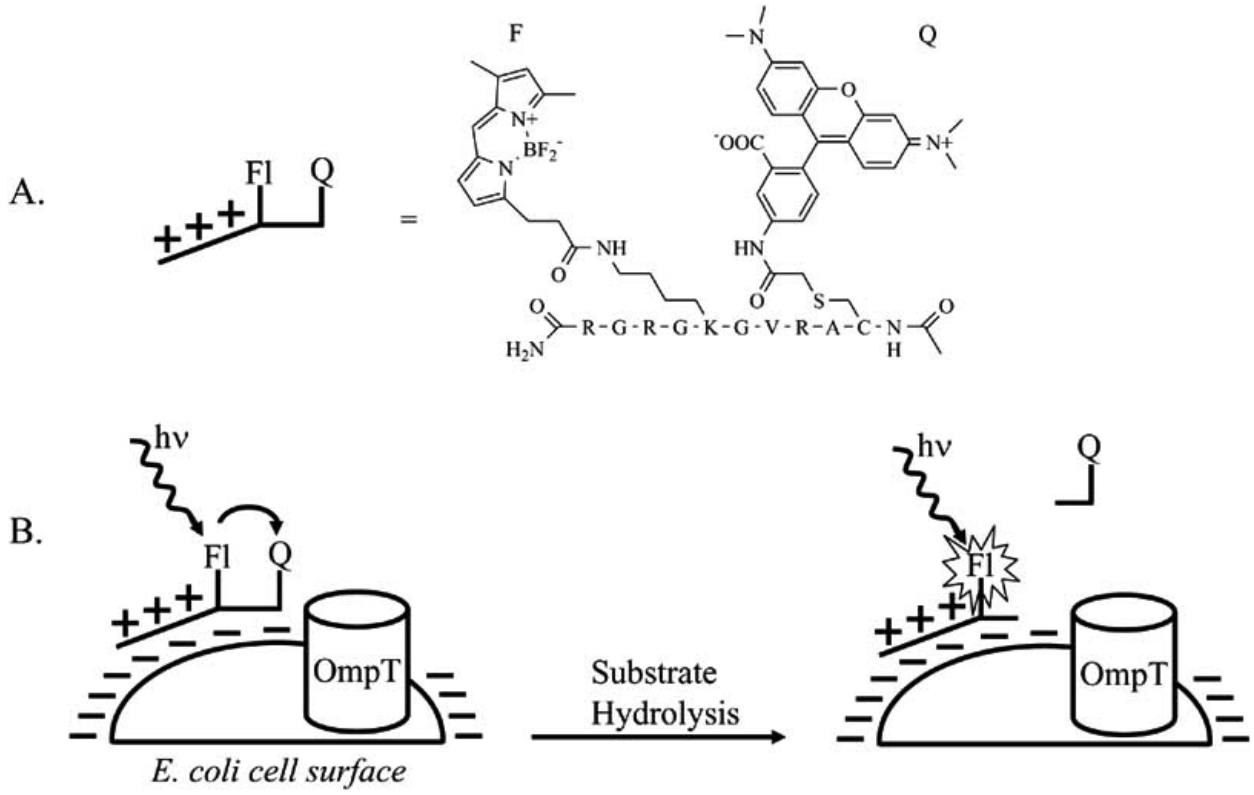

Fig. (1). Screen for catalytic activity. (A) FRET substrate designed for FACS screening for catalytic activity. (B) FRET substrate binds electrostatically to the cell surface and fluorescence emission occurs after cleavage.

hydrolyze between Arg-Arg residues [12], and the library was screened for Arg-Val cleavage. A mutant was isolated with a 60-fold improvement in catalytic activity for Arg-Val compared to the wild-type. However, the mutant also had detectable cleavage at Val-Val, Val-Gly, and Gly-Thr. The mutant had increased activity for the desired substrate, but the cleavage was still promiscuous for other sequences.

The key for the success was clever substrate design of a fluorescence resonance energy transfer (FRET) substrate. The peptide substrate contained a fluorophore (Fl), a positively charged moiety, the target scissile bond, and a fluorescent quenching partner (Q) (Fig. **1**). The FRET substrate has an overall positive charge, and it binds through electrostatic interactions to the negatively charged *E. coli* surface. Active variants can be identified since cleavage of the scissile bond results in disruption of the FRET interaction between Fl and Q. The peptide fragment containing the quenching partner diffuses away from the cell and the fluorophore remains bound to the cell surface. Hence, this approach provides a direct link with DNA sequence of the variant with catalytic activity.

The research described for OmpT was extended to screen for high catalytic activity and substrate specificity [13]. A FACS method was developed that simultaneously incorporated a selection for the desired substrate and a counter selection for the wild-type substrate. The desired substrate used was similar to the one used above which was a FRET substrate containing the Ala-Arg cleavage site. The counter selection substrate contained the wild-type cleavage site (Arg-Arg) with a single fluorophore containing three positive charges on one side of the cleavage site and an equal number of negative charges on the opposite side. This substrate cannot be preloaded to the surface since it has no net positive charge. However, cleavage of the scissile bond results in a product with a net positive charge which can bind electrostatically to the cell surface. Combining the FRET and the counter selection substrate allowed for multiparameter FACS screening and the dynamic range necessary to identify highly active and substrate specific mutants. A random mutation library was screened using this method, and a variant was isolated that had a greater than 3.0 x 10^6 reversal in selectivity for Ala-Arg over Arg-Arg. Furthermore, the enzymatic activity of the evolved OmpT variant for nonnatural substrate (k_{cat}/K_M = 1.5 x 10^5 $s^{-1}M^{-1}$) was comparable to wild-type for its preferred substrate (k_{cat}/K_M = 1.7 x 10^5 $s^{-1}M^{-1}$). Hence, the mutant had both high activity and exceptional substrate specificity.

Isolation of a catalytic antibody from a combinatorial library displayed on the surface of yeast has recently been demonstrated [14,15]. Antibodies used to catalyze chemical reactions have generated excitement as a tool for organic synthesis, and they provide a system to investigate the mechanisms of protein catalysis through the use of transition state analogs. FACS and the advances in heterologous protein display have made *in vitro* affinity maturation of antibodies more efficient. A catalytic antibody in a heterodimeric Fab format was expressed successfully on the yeast-cell, and this construct was able to catalyze the hydrolysis of a chloramphenicol monoester. A library was constructed, and FACS analysis was performed using a transition state analog tethered with a fluorophore. Nine colonies were isolated and further analyzed, and they all

hydrolyzed the chloramphenicol monoester. Only two clones showed higher catalytic activity, but the improvements were modest. Furthermore, high binding affinity did not correspond to enhanced activity. The structure/function relationships are not clear, and this study demonstrates that it is important to screen for catalysis and not binding to isolate clones with large improvements.

Enzyme screening by covalent attachment of products *via* enzyme display (ESCAPED) has recently been reported as a potential FACS based high-throughput screen [16]. The approach requires that the reaction product attach covalently to the cell surface. The screen is based on horseradish peroxidase (HRP)/tyramide reaction. Compounds linked with tyramide are substrates for HRP in the presence of hydrogen peroxide. HRP reacts with the phenolic substituent of tyramide yielding a radical intermediate. Consequently, the tyramide radical is coupled with tyrosine residues in close vicinity of HRP (Fig. **2**). Displayed EstA, an esterase from *Pseudomonas aeruginosa*, was used as a model system for the ESCAPED method. First, EstA was expressed on the surface of *E. coli* which was followed by covalent attachment of HRP to the cell surface. In order to detect hydrolases activity, the biotin-tyramide substrate contained a phenolic hydroxyl group that is ester protected. Hence, substrate attachment to the cell surfaces only occurs when an active esterase is present. This approach was demonstrated by mixing positive and control cells in a $1{:}10^6$ ratio. Approximately, 10^8 cells were assayed with the octanoic acid ester of biotin-tyramide. After the reaction, the cells were incubated with streptavidin-coated magnetic beads. After two rounds of enrichment by magnetic sorting, the isolated population displayed high esterase activity. Although it has been suggested that ESCAPE can be easily adopted for FACS, a directed evolution study has not been reported using EstA libraries. Several issues must be addressed such as diffusion of the product to surrounding cells and cell viability in the presence of hydrogen peroxide.

FACS SCREENING OF INTRACELLULAR ENZYMES

FACS screening of intracellular proteins requires that the substrate is able to enter the cell and the fluorescent product remains associated with the cell. FACS screening for enhanced ribozyme splicing activity has been demonstrated

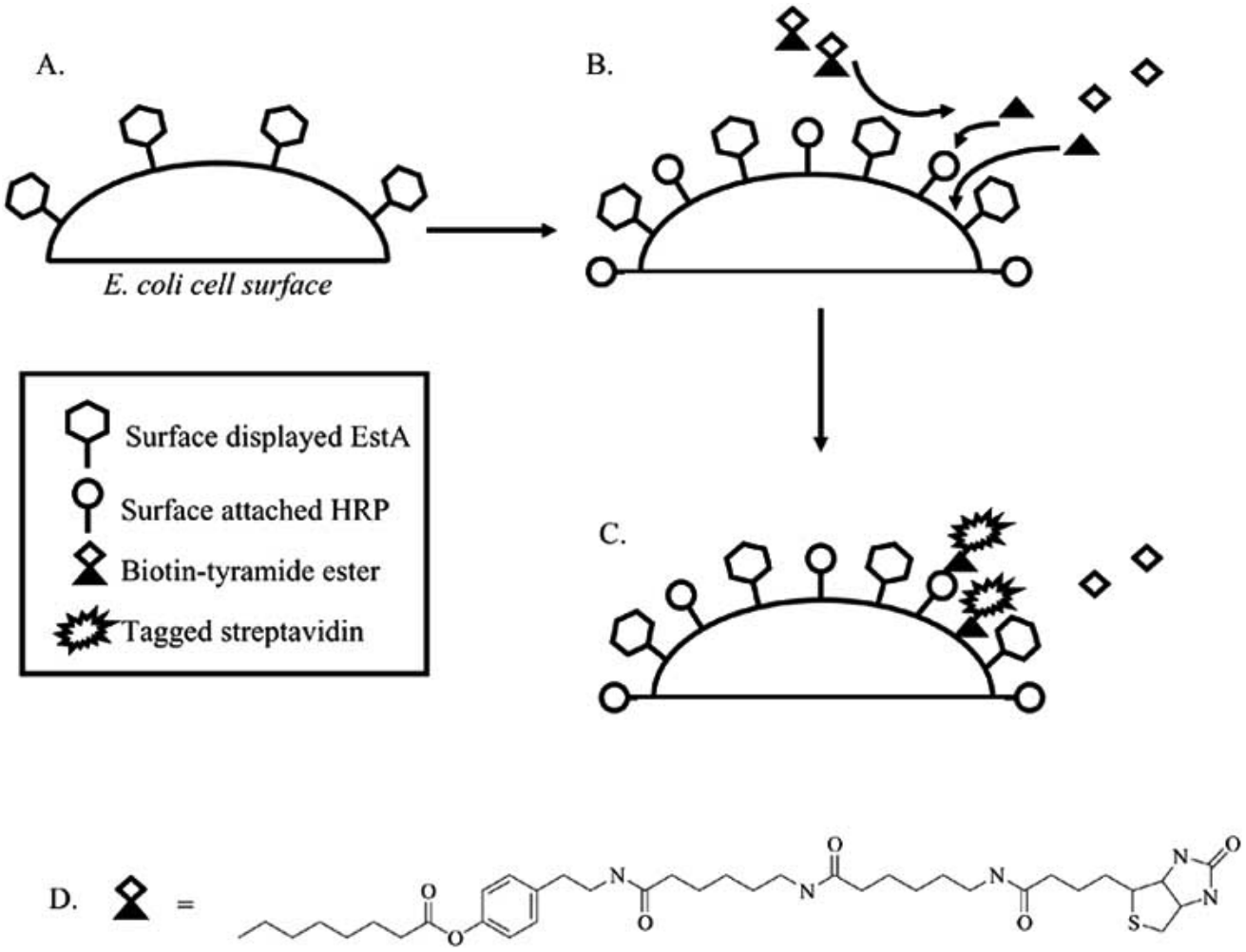

Fig. (2). ESCAPED screening strategy. (A) EstA is displayed on the cell surface. (B) HRP is covalently linked to the cell surface. Active EstA hydrolyzes the biotin-tyramide ester substrate, and HRP in the presence of H_2O_2 reacts with phenolic portion of the substrate yielding a radical that couples with tyrosine residues found on the cell surface. (C) The functionalized cells are labeled with a fluorescently tagged streptavidin. (D) Structure of the biotin-tyramide octanoic acid ester substrate used for screening.

in live mammalian cells [17]. Ribozyme variants have been shown to perform self-splicing and trans-splicing in mammalian cells, and applications include repairing genetic disorders. However, limitations exist due to sluggish activity and low yield of the final therapeutic proteins. A reporter system was developed that is amenable for high-throughput screening and flow cytometry. The system is based on the enzyme β–lactamase (Bla). To detect Bla activity, a fluorogenic substrate (Fig. **3**) was designed to pass through the cell membrane and the fluorescent product remains in the cell. Constructs were engineered containing the self-splicing *Tetrahymena thermophila* group I intron ribozyme inserted into the open reading frame of the mRNA of TEM-1 Bla. This approach was used to screen ribozyme L1 loop variants for increased Bla expression, and a variant was isolated with a 4-fold increase in activity. This study also showed that the L1 loop of the *Tetrahymena* ribozyme plays an important role for cis-splicing demonstrating the use of FACS to obtain structure/function relationships.

A FACS screen has been described as a potential tool for directed evolution of active aldolase antibodies that are expressed intracellularly [18]. The approach did not use a transition state analog functionalized with a fluorophore. Instead, a substrate was designed and synthesized to directly detect catalytic activity. The substrate (Fig. **4**) has low cellular background, and the attached fluorophore has a high quantum yield. The bulky fluorophore is tethered to a long linker away from the reactive site, and the product fluorophore is retained in the cell. Reaction of the antibody with the substrate resulted in a tandem retro-aldol retro-Michael reaction, and the linker is cyclized releasing urea and 5-chloromethylfluorescein. Finally, retention of the fluorescent product is achieved because the chloromethyl moiety reacts with nucleophiles naturally present inside the cell. To demonstrate this approach, hybridoma cells expressing active antibody or non-catalytic antibody were mixed with the screening substrate and two distinct populations resulted with different fluorescence intensities. Subsequently, it was shown that cells expressing catalytic active antibodies can be sorted from a large excess of cells expressing inactive antibodies. The study also determined that active catalyst can be identified from transfected mammalian cells expressing the active antibodies using flow cytometry. This method can potentially be used to analyze a plasmid-encoded library introduced and expressed in mammalian cells.

FACS screening of Cre recombinases from bacteriophage P1 with altered DNA-recognition sites has recently been reported, and the assay was based on a fluorescent reporter system [19]. Cre catalyzes the recombination at DNA sequences known as *loxP*. Cre is able to catalyze DNA integration, excision, or rearrangement depending on the location and orientation of the two participating *loxP* sites. Cre has been described as the universal reagent for genome

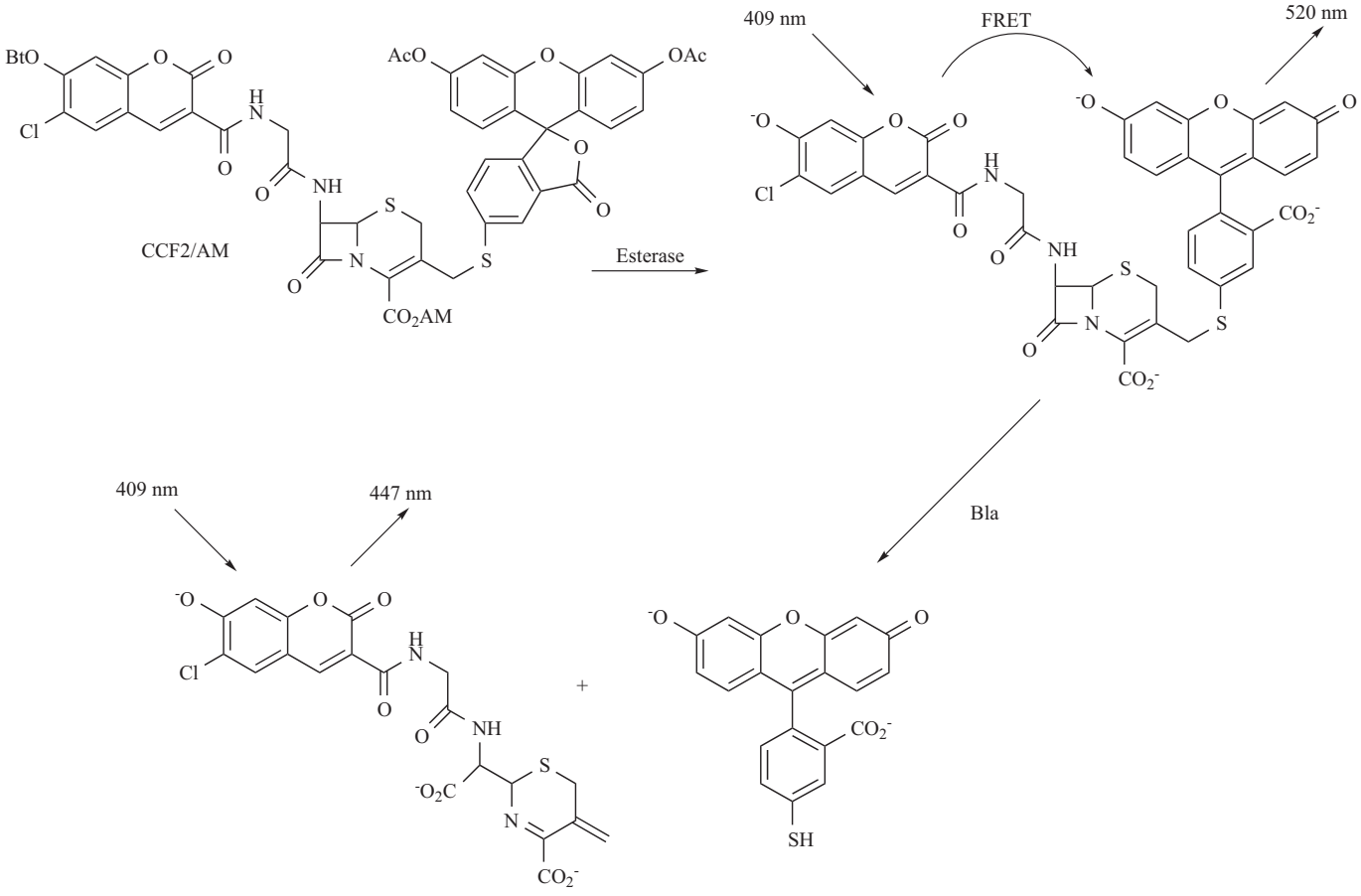

Fig. (3). Screening substrate (CCF2/AM) for Bla activity in living cells. CCF2/AM is able to pass through the cell membrane and intracellular esters yields CCF2. In the presence of Bla, the FRET interaction in CCF2 is disrupted and emission shifts from 520 nm to 447 nm.

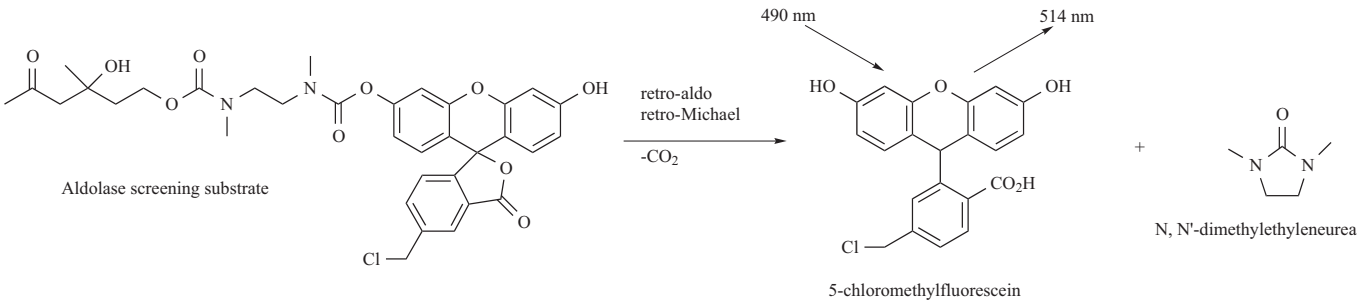

Fig. (4). Aldolase antibody reaction with the screening substrate. A tandem retro-aldo retro Michael reaction occurs which results in releasing urea and 5-chloromethylfluorescein.

tailoring [20] and applications include introducing point mutations, gene replacement and deletions, switches for gene activation or inactivation, and chromosomal engineering. The Cre recombination system requires that the target DNA region contains *loxP* sites restricting its use for broad applications. Hence, the ability for Cre to recognize artificially introduced recombination sites would expand the uses by targeting specific regions of genomic DNA for recombination or deletion. A gene library of Cre was constructed and screened for recombination at designed *loxP* sites. Library construction was guided by the three-dimensional structure and the amino acids chosen to be randomized were in a close proximity to the mutated base pairs of the novel *loxP* sites.

The FACS screening strategy based on a fluorescent reporter system allows for positive and negative selections. The positive selection identifed Cre mutants that recognize the designed *loxP* sites, and the negative selection distinguished Cre variants that are unable to recognize the wild-type *loxP* site. The *loxP* sites were designed such that wild-type Cre was not active. The screening system included a recombinase plasmid (pR) containing the Cre gene and a recombination reporter plasmid (pS) for FACS. Plasmid pS included two *loxP* sites, two green fluorescent protein variants (EYFP and GFPuv), and a promoter upstream of the EYFP and one of the *loxP* sites. Plasmid pS would reversibly recombine resulting in reorientation of the GFPuv gene downstream the promoter, and a change of expression from EYFP to GFPuv would occur. Cells expressing active Cre would result in rapid and reversible plasmid rearrangement resulting in equal expression of EYFP and GFPuv. On the other hand, cells expressing inactive Cre would only express EYFP because rearrangement would not occur. After five rounds of alternating positive and negative sorting, a Cre variant was identified that recognized the designed *loxP* site selectively. FACS screening with only a positive selection resulted in recombinases with high activity for the designed *loxP* site, but they were also active with the wild-type *loxP* site. These results stress that it may be necessary to integrate a negative selection to identify highly active and selective mutants.

Incorporating artificial amino acids at specific sites in a protein *in vivo* would be a powerful tool to study structure/function relationships and to create novel proteins for biotechnological applications. The previously reported positive-negative screening strategy was cumbersome and required moving the synthetase libraries between cells containing two different reporter plasmids, and the need to repeatedly transform bacteria limited the rate of evolution. Hence, a simplified system was developed incorporating FACS for evolution of aminoacyl-tRNA synthetase specificity [21].

A single genetic reporter system was constructed to allow positive and negative selections. The plasmid (pREP/YC-JYCUA) contained the gene for T7 RNA polymerase with amber codons at tolerant sites, and the product directed the expression of GFPuv. Plasmid pREP/YC-JYCUA is compatible with another plasmid that contained members of tyrosyl-tRNA synthetase library. The system was utilized to explore the evolvability of the synthetase to incorporate amino-, isopropyl-, and allyl-containing tyrosine analogs. The positive screen does not distinguish between clones that accept the nonnatural amino acid and mutants that use at least one of the naturally occurring amino acids. FACS screening eliminated synthetase variants that accepted the natural amino acids. Synthetases capable of accepting the artificial amino acids were successfully isolated.

A high-throughput screen for porphyrin metal chelatases has been reported [22]. A porphyrin consists of four pyrrole rings linked by methene bridges. Porphyrins have potential applications in biocatalysts, electronic technologies, and therapeutic agents. Structural and functional diversity can be achieved by varying the substituents on the tetrapyrrole scaffold. Total chemical synthesis or derivatizations of naturally occurring metalloporphyrins are common methods to obtain these compounds. However, biosynthetic-pathway engineering combined with rational and evolutionary protein design approaches offers an alternative strategy in accessing tetrapyrrole diversity. Recently, a system has been developed to overproduce porphyrins in *E. coli* [23]. However, the overall production of the heme was limited due to the low *in vivo* activity of ferrochelatase which is responsible for insertion of Fe(II) into protoporphyrin IX. Ferrochelatase was targeted for directed evolution since it appeared that insertion of the metal ion was limiting the overall yield. A FACS screen was developed to discriminate cells containing protoporphyrin IX or heme. Protoporphyrin IX has a high fluorescence intensity compared to heme, and the population of cells with intact heme results in decreased fluorescence. A library was constructed and screened, and the selected mutants displayed a 1.4-2.4 fold higher catalytic turnover compared to the wild-type enzyme. The amino acid substitutions were mapped on the X-ray structure to determine the structure/function relationship. All of the amino acid substitutions were located on the surface of the protein expect for two. One was found on the “lid” that the

covers the substrate binding pocket and the other is situated in the active site. However, the effect of the amino acid substitutions is not clear and requires additional experiments.

Evolution of highly active glutathione transferases (GSTs) by homology independent recombination has recently been accomplished [24]. The role of GSTs is cellular detoxification by conjugating reactive electrophilic compounds to glutathione (GSH). A library recombining rat GSTθ-2-2 (rGSTT2-2) and human GSTθ-1-1 (hGSTT1-1) was to be constructed. These enzymes exhibit only 54.3% overall amino acid identity. The three-dimensional structure is similar, and they have unique electrophilic substrate selectivities. These enzymes served as a suitable model system to investigate homology-independent recombination.

In order to create the enzyme library, a new method was developed that did not rely on DNA shuffling methods. DNA shuffling by sexual PCR has proven to be successful in creating chimeric genes with improved and novel activities [25-27], and these variants are based on homologous recombination between closely related parental sequences. On the other hand, the recombination of distantly related sequences lacks diversity because large fractions of the library are unshuffled parental sequences. A new method was developed to create libraries containing multiple non-homologous crossovers [28].

A chimeric library was created and screened using rGSTT2-2 specific fluorogenic substrate, 7-amino-4-chloromethyl-coumarin (CMAC). CMAC does not fluorescence until it is conjugated with free thiols such as GSH. One isolated clone, SCR23, had 3.5- and 300-fold higher k_{cat} values for CMAC relative to rGSTT2-2 and hGSTT1-1, respectively. SCR23 contained helices from rGSTT2-2 into the hGSTT1-1 scaffold which is part of substrate binding domain. Furthermore, the variant catalyzed the conjugation of GSH to ethacrynic, and this activity is not detectable in both parental proteins. These results demonstrate that novel and highly active enzymes can be identified from libraries containing a large fraction of homology-independent crossovers.

FACS SCREENING IN COMPARTMENTS

In vitro compartmentalization (IVC) has been developed to associate genotype-phenotype relationships [29]. IVC is a cell free system based upon creating microenvironments consisting of water-in-oil emulsions with reaction volumes of approximately 5 fl. Hence, a 50 µl would contain approximately 10^{10} compartments or reactions resulting in an economical system for screening.

Directed evolution of phosphotriesterase (PTE) with higher activity has recently been reported using IVC [30]. Phosphotriesterase can degrade organophosphates pesticides and nerve agents. The screening procedure is outlined in Fig. **5**. First, the microbead-displayed gene-protein complex is constructed in the initial emulsion, and the beads were re-compartmentalized in a second emulsion. Next, the substrate which is tethered with a caged-biotinylated substituent was added to the oil phase which diffuses into the aqueous compartment. After the reaction time, the solution is irradiated to uncage the biotinylated product or substrate which binds to the streptavidin-coated bead. Finally, the emulsion is disrupted and the beads are labeled using fluorescently labeled antibodies specific for the product, and flow cytometry is used to isolate active enzymes.

A PTE library was constructed by randomizing amino acids that compose the substrate binding pocket based on the structure. PTE is already very active with paraoxon (k_{cat} = 2280 s^{-1}, k_{cat}/K_M = 6.2 x 10^7 $M^{-1}s^{-1}$), and it was screened for increased activity. The screening substrate included paraoxon tethered to a caged-biotinylated substituent. A clone was isolated from a library containing 3.4 x 10^7 members that had a 63-fold improvement in k_{cat} and slightly higher k_{cat}/K_M (1.8 x 10^8 $M^{-1}s^{-1}$) for paraoxon compared to the wild-type PTE.

IVC has several advantages compared to using microbes for high-throughput screening for enzymatic activity. First, transformation and cloning is unnecessary since the gene is compartmentalized *in vitro*. Next, there is greater control of the reaction conditions. For example, any buffer and temperature up to 99 ^oC can be used; whereas, reactions *in vivo* or with lysed cells occurs in a complex environment. Furthermore, the screens are based on turnover of soluble substrates.

IVC by double emulsions has been developed for gene enrichment using flow cytometry [31]. In contrast to the method above, this approach does not depend upon attachment of the biocatalytic product to a microbead. The w/o emulsions described above has a continuous oil phase which is not compatible with flow cytometry. Hence, double (w/o/w) emulsions were developed to create an external aqueous shell surrounding the primary w/o emulsion. The result is an external aqueous phase that does not disturb the internal aqueous droplet. Consequently, genes are transcribed and translated in the primary w/o emulsion, and the conversion to w/o/w emulsion makes the system suitable for high-throughput screening using flow cytometry. These emulsions were shown to withstand forces applied during screening and no leakage between emulsions occurred. The potential of using w/o/w for high-throughput screening was demonstrated by sorting positive emulsions from negative emulsions. First, two separate w/o emulsions were constructed. The positive emulsions contained *fol*A gene and FITC-BSA and the negative w/o emulsion consisted of a gene of different length (M.*Hae*III) with no fluorescent marker. The genes were amplified and tagged at the 5' end with biotin. Next, the positives were mixed with the negatives in a 1:100, respectively, and re-emulsified to create the w/o/w emulsion. The resulting w/o/w emulsions were sorted with flow cytometry, and enrichment of 38.5 fold was achieved. The sorted emulsions were disrupted and the genes were bound to streptavidin-coated magnetic beads. PCR analysis shows that enrichment of approximately 30-fold was obtained. These results demonstrate that little or no mixing of either DNA or FITC takes place during the process of forming w/o/w emulsions.

This system has been suggested to be useful for high-throughput screening of enzymes using fluorogenic substrates that do not necessitate attachment or restriction. Potential applications of w/o emulsions would be to compartmentalize protein displayed libraries on cell surfaces, phage, ribosome, or mRNA-peptide fusions. Essentially, w/o/w emulsions have the potential to be used as a high-

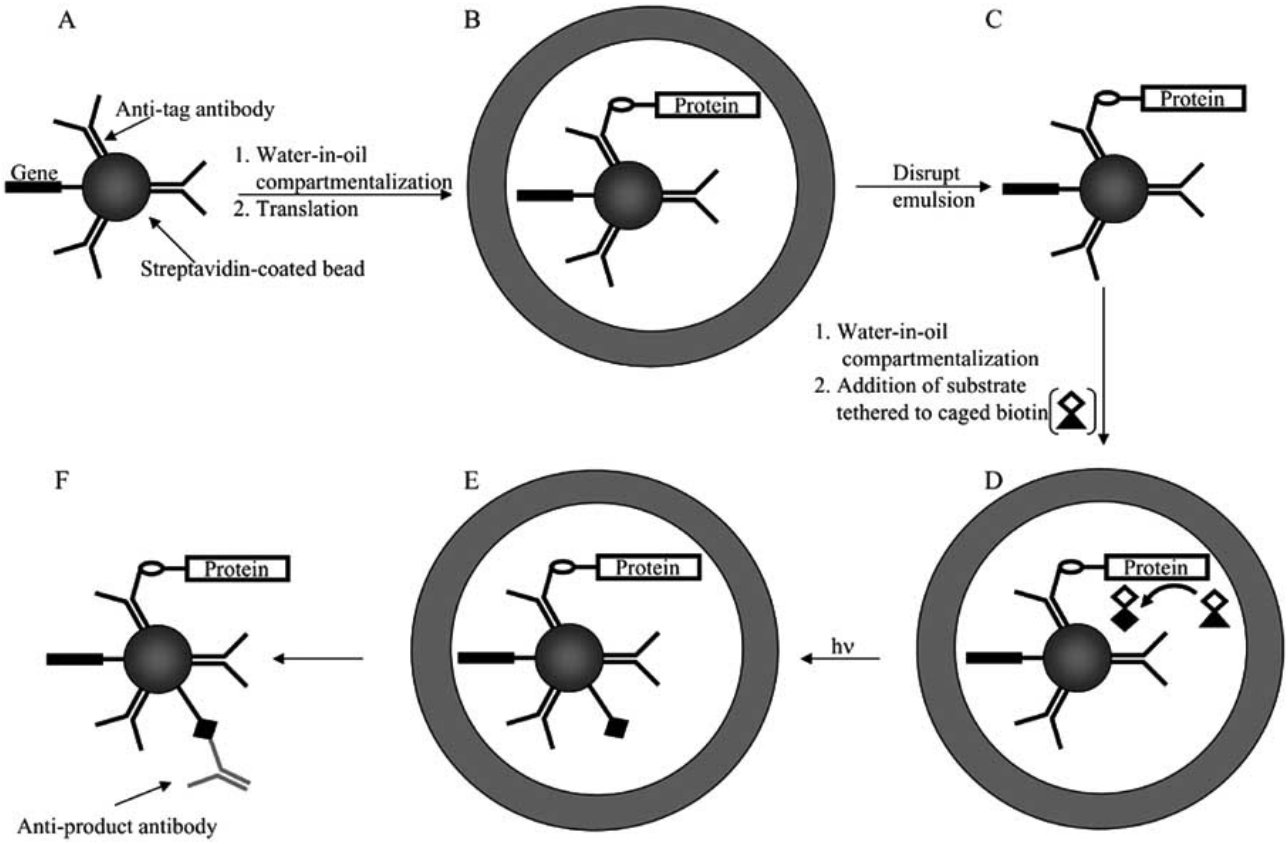

Fig. (5). Construction of microbead-display libraries and high-throughput screening using flow cytometry. (A) Generation of microbead displayed libraries: A gene library is created containing a common epitope tag and it is attached to streptavidin-coated beads carrying antibodies specific for the epitope tag. (B) The beads are compartmentalized in a water-in-oil emulsion, and the gene library is transcribed and translated. The translated enzyme is attached to the corresponding bead that encodes the gene. (C) The emulsion is destroyed and the beads are isolated. High-throughput screening for enzymatic activity using flow cytometry. (D) The beads are compartmentalized in a water-in-oil emulsion, and the substrate tethered with a caged-biotin is added. Substrate turnover occurs only in compartments that contain beads with active enzyme. (E) Irradiation of the emulsion uncages biotin. Consequently, the product becomes attached to the bead. It should be noted that unreacted substrate is also attached to beads in compartments with inactive enzyme. (F) The emulsion is disrupted and the beads are incubated with anti-product antibodies that are fluorescently labeled.

throughput tool without requiring a physical link between the product and the displayed protein.

In conclusion, the advantage of FACS is that larger libraries can be screened compared to tradition methods. FACS screens for improved binding affinity or altered substrate specificity has been widely used and very successful. On the other hand, using flow cytometry to screen for enzymatic activity has been met with limited success, but the technology is rapidly advancing as clever systems are developed. In short, FACS is emerging as a general laboratory tool for protein engineering and studying structure/function relationships. FACS will also be useful to compare and contrast different directed evolution methods, and important statistical information can be quantified.

NOTE ADDED IN PROOF

Recently, directed evolution of serum paraoxonase (PON1) was successful using w/o/w emulsions. Single bacterial cells, each expressing a unique PON1 mutant, were compartmentalized in a w/o/w emulsion. FACS screening identified a variant with 100-fold improvement in thiolactonase activity [32]. Furthermore, a cell-free translation system has also been used with w/o/w emulsions for laboratory evolution of novel β-galactosidase mutants [33]. Together, these papers demonstrated that w/o/w emulsions can be employed for *in vivo* and *in vitro* systems.

ABBREVIATION

Arg	=	Arginine
Bla	=	β–Lactamase Bla
CMAC	=	7-Amino-4-chloromethyl-coumarin
DNA	=	Deoxyribonucleic acid
ESCAPED	=	Enzyme screening by covalent attachment of products *via* enzyme display
EstA	=	Esterase from Pseudomonas aeruginosa
EYPF	=	Enhanced yellow fluorescent protein
F	=	Fluorophore
Fab	=	Fragment antigen binding
FACS	=	Fluorescence activated cell sorting
FITC	=	Fluorescein isothiocyanate
FRET	=	Fluorescence resonance energy transfer

GFPuv = Uv optimized green fluorescent protein
GSH = Glutathione GSH
GST = Glutathione transferase
HRP = Horseradish peroxidase
IVC = *In vitro* compartmentalization
PCR = Polymerase chain reaction
PTE = Phosphotriesterase
Q = Quenching partner
w/o = Water-in-oil emulsions
w/o/w = Double emulsions
Val = Valine

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