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Generation of New Enzyme Catalyst by Directed Evolution and Functional Tuning

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Both the directed evolution and functional tuning are a new process for promising biocatalysts that viable in industrial, also pharmacological, applications. The technique does not require a resolved 3D structure and thus comprehensive information about the structure and function of proteins as required conventional protein engineering. They mimic the process of natural evolution in a test tube by rational and/or irrational approaches that progressed solely or combined with conventional technique when required. Directed evolution and functional tuning, therefore, generated an enzyme with enhanced performance both *in-vitro* and *in-vivo* conditions.

Biological catalysts, mainly enzyme, in general are attractive for industrial purposes because they are efficient and selective in the chemistries they execute. Enzymes and proteins precise functionally had a good performance and thus well matched with most reactions found naturally or exploited in industry. However, naturally occurring enzyme often lack properties necessary for practical applications. Hence, available natural enzyme sources, such as enzyme (or protein) itself or putative genetic material, are being tailored to fulfill increasing demands for new biocatalysts that designed to break the typical barriers. Although a lot of approaches continued to prepare the novel enzyme by using the natural enzyme with known role, the intensive works that generated a novel function unable to screen from natural sources will be guided us to new fields of pharmaceutical and fine-chemical biocatalyst. In line with this scope, we currently are pursuing two studies; URFs engineering for assigning or adapting functions by directed evolution; Tailoring bi- or muliti-functional enzymes by tuning an artificial fusion protein.

Assigning or adapting functions by directed evolution of URFs. URF, unidentified open reading frames, are found ubiquitously from various genome sequences completed to date. Much remained portion of open reading frames are also predicted as putative enzymes by sequence-based alignment tools, mainly due to the absence of established genetic data or lack of attention to the physiological role of these genetic materials. Although the current concepts that assigned an exact function in-vivo are partly insufficient, the engineering on these materials are feasible for generating new enzymes by directed evolution and then functional tuning, because

that the materials can be easily classified into a group relative structurally to known family of enzymes. Such studies, therefore, that adopted some approaches to define a close or remote homology of URFs to a family of enzymes, are conducted here. Consequently, several URF candidates that showed structural clues for possible function are expressed to confirm a function or engineered to address a novel function.

Tailoring bifunctional enzymes by tuning an artificial fusion protein. In the field of molecular biology and biotechnology, enzymes or proteins possessing two or more combined activities, along with appropriate stability, have found a wide application. Although the natural huge diversity of the enzymes has provided some candidates that have evolved to possess bi- or multi-functional activity, most fusion enzymes have resulted from the *in-vitro* fusion of individual enzymes based on evolutionary traits and a well-defined structure. Artificial fusion proteins, simple generated by either end-to-end fusion or tethering with a linker of whole genes or restricted domains, have shown comparable performance with each enzyme in a concerted fashion. However, in general, functional and structural instability of artificial fusion has limited its practical use. We attempted to construct an artificial fusion enzyme composed of N-carbamylase and D-hydantoinase, in an effort to produce optically pure D-amino acids by the single polypeptide that mediated a sequential reaction, and drastic improvement in structural and functional stability by tuning with directed evolution was achieved.

Both approaches briefly noted here is an example showing that artificial adaptation or fusion of an open reading frame broadens the application range of natural genes encoding either a functional protein or a putative enzyme.