Supplementary methods 2. Full protocol for systematic remodeling of plasticity residues for improved protein functions.

The methodology developed herein, systematic remodeling of plasticity residues, is based on the empirical realization that (1) proteins with more specific and active functions could divergently evolve from proteins with promiscuous functions[1-5], (2) mutations at the plasticity residues could significantly drive molecular evolution[6], and (3) most of the substitutions in plasticity residues can additively affect the protein functions[7]. Hence, if several important plasticity residues could be identified in the targeted proteins with promiscuous functions, it would be possible to specify each of function. The procedures for the practical applications are described as follows.

1) Selecting proteins with promiscuous function

Promiscuous function is thought to be very important for organisms to adapt rapidly changing environment and for proteins to evolve divergently to acquire more specific and active functions[1-5]. As such, it is essential to select the proteins with promiscuous functions. Since it is well known that many proteins have promiscuous functions to some degree, many of interesting functions could be found as minor function of certain proteins.

2) Identification of plasticity residues

Plasticity residues are defined as those that primarily govern enzyme specificity (e.g. distinguish some reactions from others). These residues should be considered separately from the residues primarily important for catalytic activity. For example, in the case of terpene cyclases, the aspartates in the two aspartate rich motifs and the two arginine residues located in the upper part of the active site are thought to be very important for the substrate binding and initiation of the carbocation reactions. These residues are generally conserved in all terpene synthases, and mutations to these residues often result in a significant loss of overall specific activity. Thus, these residues are more essential for catalysis in this class of enzymes, even though mutations to these residues affect product selectivity. As for the substrate specificity, residues that distinguish one substrate from other substrates can be referred as plasticity residues.

Plasticity residues can be identified by various methods: structural analysis, random mutagenesis, and phylogenetic analysis have often been utilized to predict whether a particular residue is plasticity residue or not. We utilized the modeled structure as a guide to predict plasticity residues. The predicted plasticity residues are then subjected to saturation mutagenesis, followed by functional analysis. If the residues of interest are plasticity residues, a gradual shift of one function to another over wide range can be observed as the type of the amino acid changes. The product profile

resulting from each mutation is normalized to that of wild type and summarized as described in the 'Methods' section.

(3) Systematic remodeling of plasticity residues

Systematic remodeling is based on the assumption that there is no interaction between plasticity residues; hence, the effect given upon the mutation of each plasticity residue is the same for both wild type and mutants. Validation of this assumption is based on the empirical observation (throughout the history of protein engineering) that most mutations additively affect the protein functions[7]. The mutations were introduced to minimize the ω value. The ω value is calculated based on the algorithm described in 'Methods' section.

References

1. Jensen, R. A. Enzyme recruitment in evolution of new function. *Annu Rev Microbiol* **30**, 409-25 (1976).
2. O'Brien, P. J. & Herschlag, D. Catalytic promiscuity and the evolution of new enzymatic activities. *Chem Biol* **6**, R91-R105 (1999).
3. Copley, S. D. Enzymes with extra talents: moonlighting functions and catalytic promiscuity. *Curr Opin Chem Biol* **7**, 265-72 (2003).
4. James, L. C. & Tawfik, D. S. Conformational diversity and protein evolution--a 60-year-old hypothesis revisited. *Trends Biochem Sci* **28**, 361-8 (2003).
5. Gerlt, J. A., Babbitt, P. C. & Rayment, I. Divergent evolution in the enolase superfamily: the interplay of mechanism and specificity. *Arch Biochem Biophys* **433**, 59-70 (2005).
6. Aharoni, A. et al. The 'evolvability' of promiscuous protein functions. *Nat Genet* **37**, 73-6 (2005).
7. Brannigan, J. A. & Wilkinson, A. J. Protein engineering 20 years on. *Nature Reviews Molecular Cell Biology* **3**, 964-970 (2002).